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(54) Title: DEGRADABLE CROSS-LINKING AGENTS AND CROSS-LINKED NETWORK POLYMERS FORMED THEREWITH

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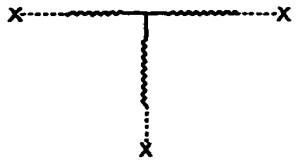

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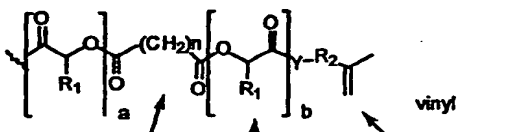

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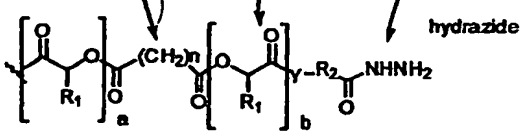

(57) Abstract: Degradable cross-linkers which are used to form polymer networks which degrade under aqueous conditions are described. These cross-linkers comprise a monodispersed central polyacid, monodispersed monomeric or monodispersed oligomeric degradable regions and an optional water soluble regions. These monomers are preferably polymerized using free radical or condensation polymerization. Degradation occurs at degradable regions such as ester linkages after cross-linking polymer filaments, and results in soluble polymer filaments which may be cleared from the body. Preferred applications of these materials include, for example, controlled release of drugs and cosmetics, tissue engineering, wound healing, hazardous waste remediation, metal chelation, swellable devices for absorbing liquids and the prevention of surgical adhesions.

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DEGRADABLE CROSS-LINKING AGENTS AND CROSS-LINKED NETWORK POLYMERS FORMED THEREWITH

BACKGROUND OF THE INVENTION

1. Field of the Invention.

The present invention relates to novel cross-linking agents, more particularly to novel biodegradable cross-linking agents. Earlier use of cross-linking agents in a variety of fields involving proteins, carbohydrates or polymers is well established. Even biodegradable cross-links have previously been prepared and utilized. However, none before have utilized particular and advantageous cross-linker designs of the present invention.

Within the pharmaceutical, agricultural, veterinary, and environmental industries, much attention has been directed to the applications of biodegradable polymers. The Oxford English Dictionary defines biodegradable as: "susceptible to the decomposing action of living organisms especially bacteria or broken down by biochemical processes in the body." However, due to the advent of the widespread use of polyhydroxyacids as degradable polymers, this definition should be extended to include non-enzymatic chemical degradation which can progress at an appreciable rate under biologically relevant conditions (the most relevant condition being water at pH 7; 100 mM salt and 37°C). Thus, the meaning of the term *biodegradation* can be broadened to include the breakdown of high molecular weight structures into less complicated, smaller, and soluble molecules by hydrolysis or other biologically derived processes.

In the biomaterials and/or pharmaceutical areas, there is great interest in the use of biodegradable materials *in vivo*, due to performance and regulatory requirements. However, most of the reports on biodegradable materials have focused on linear water-insoluble

hydroxyacid polyesters. Much less work has been done on biodegradable network polymers which are cross-linked. Therefore, due to the unique properties of network polymers, or more specifically a network of cross-linked polymer filaments, it is to be expected that biodegradable networks will find many new and important applications.

Biodegradable polymers

Much work has been accomplished in the last 20 years in the area of hydrophobic biodegradable polymers, wherein the biodegradable moieties comprise esters, lactones, orthoesters, carbonates, phosphazines, and anhydrides. Generally, the polymers made of these biodegradable linkages are not water soluble and therefore in themselves are not amenable for use in systems where water is required, such as in hydrogels.

Since the mechanism of biodegradation in these polymers is generally through the action of the hydrolytically-active components of water (hydronium and hydroxide ions), the rate of hydrolytic scission of the bonds holding a polymer network together is generally pH sensitive, with these moieties being susceptible to both specific-acid catalyzed hydrolysis and specific base-induced hydrolysis. Other factors affecting the degradation of materials made of these polymers are the degree of polymer crystallinity, the polymer volume fraction, the polymer molecular weight, the cross-link density, and the steric and electronic effects at the site of degradation.

Degradable Network Structures

Biodegradable networks of cross-linked polymer filaments are prepared by placing covalent or non-covalent bonds within the network structure that are broken under biologically relevant conditions. This involves the use of two separate structural motifs. The degradable structure is either placed into (i) the polymer filament backbone or (ii) into the

cross-linker structure. The method described herein creates a degradable structure through placing degradable regions in the cross-linking domain of the network.

One of the first occurrences of degradable hydrogels was published in 1983 by Heller. This system contains a water soluble linear copolymer containing PEG, glycolylglycolic acid and fumaric acid linkages. The fumaric acid allowed the linear polymer to be cross-linked through free radical polymerization in a second network forming polymerization step, thus creating a cross-linked network of polymer filaments which could degrade through hydrolysis of the glycolic ester linkages. This is an example of creating degradable linkages in the polymer backbone.

Biodegradable Cross-linkers

The first truly degradable cross-linking agents were made from aryl diazo compounds for delivery of drugs in the digestive tract. The diazo moiety is cleaved by a bacterial azoreductase which is present in the colon. This has been used to create colon specific delivery systems (Brondsted *et al.* & Saffran *et al.*). Another biodegradable cross-linking agent appears in the work of Ulbrich and Duncan where a bis-vinylic compound based on hydroxyl amine was synthesized. Hydrogels made from this degradable cross-linker were shown to undergo hydroxide induced hydrolysis of the nitrogen-oxygen bond.

Hubbell *et al.* have made hydrogels composed of macromonomers composed of a central PEG diol which was used as a bifunctional alcohol in the tin octanoate catalyzed transesterifying ring opening polymerization of lactide to give a bis-oligolactate PEG. This compound was then reacted with acryloyl chloride to give a macromolecular cross-linker which could be formed into a homo-polymer interpenetrating network of PEG and oligolactylacrylate through free radical polymerization (Pathak *et al.*). Hubbell mostly

intended these compounds for use as photopolymerizable homo-polymers useful to prevent surgical adhesions.

A second solution to this problem has been recently reported in the work of Van Dijk *et al.* which is the first report of a biodegradable cross-linking macromonomer composed of alpha-hydroxy esters (Van Dijk-Wolthius *et al.*). This work combines natural polymers with synthetic polymers in an interpenetrating network. This group functionalized dextran with oligo-alpha hydroxy acid domains which were end capped with vinyl regions that were polymerized into biodegradable networks via free radical polymerization.

The most recent report of a biodegradable cross-linking agent was one designed to undergo enzymatic degradation. This cross-linker is composed of a centro-symmetric peptide terminated by acrylamide moieties with a central diamine linking the two ends (Kurisawa *et al.*). This report is related to the invention described herein in that the property of biodegradability is built into the polymer network by first synthesizing a small symmetrical or small asymmetrical cross-linker which can undergo cleavage, then incorporating this in a polymer network.

Properties of Degradable Gels: Swelling and Porosity

Since degradability is a kinetic effect, the properties of degradable gel networks are similar to those standard gel networks, except they change with time. The two main properties that are exhibited by degradable hydrogel networks are swelling and network porosity that increase with time as the network degrades.

The main feature observed with degradable cross-linked polymer networks in solvents which cause them to swell is that the polymer network swells as it degrades. This is because network degradation results in a decrease in cross-link density. As the cross-link density decreases there is more available volume for solvent within the network. The solvent

increasingly permeates the network structure, driven by a favorable thermodynamic mixing of solvent with the polymer network.

Important uses envisioned for degradable gels are as controlled drug delivery devices and as degradable polymers for other *in vivo* uses. These devices are able to change from a high viscosity material (gel) to a lower viscosity soluble material (sol). The resulting water soluble linear polymer can then be readily transported and excreted and/or degraded further.

Degradable hydrogel networks offer the opportunities to effect the diffusitivity of materials bound in the hydrogel network, because as the network degrades the diffusion coefficient of molecules in the network increases with time thus facilitating the release of materials locked within the polymer network (Park). Moreover, because the hydrogel network structure itself is of such a high molecular weight, transport of the hydrogel network out of the body or environment is slow. This is especially true *in vivo* where non-degradable implanted hydrogel networks can remain in the body for many years (Torchilin *et al.*). Therefore, such devices would be more useful if they could be made of a high molecular weight polymer that would degrade into smaller molecular weight components after the device has performed its task and then could be excreted through normal routes of clearance. Since excretion of polymers is molecular weight-dependent (Drobnik *et al.*), with the preferred route being through the renal endothelia (Taylor *et al.* & Tomlinson), the chains making up the polymer backbone should be between 10 and 100 kDa. Because the material is engineered to degrade into excretable parts, biodegradable hydrogel networks offer increased biocompatibility.

Biodegradable Network Polymers as Controlled Release Depots

Biodegradable network polymers can be used as carriers for biologically active substances. These include DNA, RNA, proteins, peptides, saccarides, poly-saccarides, hormones, anti-cancer agents, antibiotics, vaccines, herbicides, insecticides and cell suspensions. The hydrophilic or hydrophobic polymer network can act as a stabilizing agent for the encapsulated species and as a means to effect a controlled release of the agent into the surrounding tissue, systemic circulation, or environment. By changing the size of the depot, the degree of porosity, the hydrophilicity/hydrophobicity of the network, and the rate of degradation (through modification of the degradable regions in the polymer network) controlled release depots with a variety of release characteristics can be fashioned for applications in the medical, diagnostic, agricultural, and environmental areas.

Biodegradable Network Polymers as Water Adsorbents

Owing to the ability of hydrophilic network polymers to adsorb water, biodegradable versions of these networks may prove to have many uses in items for example, sanitary napkins, wound dressings, and diapers. When these materials are used in consort with other degradable materials a completely biodegradable and disposable product could be produced. In fact, a literature search found that Sikes, (U.S. Patent No. 5,773,564) has developed adsorbant materials from cross-linked polyaspartate.

Biodegradable Network Polymers as Adhesives

There is a great need for biodegradable adhesives and sealers in surgery and elsewhere. Synthetic polymers have been used as adhesives in surgery with the cyano acrylate esters being the most commonly cited. Recent reports using biodegradable networks as sealants in dentistry and orthopedics have displayed the utility of biodegradable polymers (Burkoth). There the use of a biodegradable cross-linking monomer (bis-

methacrylated diacid anhydride) which has been photopolymerized is envisioned for use in dentistry. In Burkoth a hydrophobic network-forming monomer is photopolymerized *in situ* to form a mechanically stable and non-swellable bonding material. Degradability would be a desirable property for any short term application though undesirable for long term applications.

Use of Biodegradable Polymers in Drug Delivery

Since most synthetic biodegradable polymers are not soluble in water, a hydrophilic drug is formulated in these polymers by a dispersion method using a two phase system of water (containing drug) and organic solvent (containing the polymer). The solvent is removed by evaporation resulting in a solid polymer containing aqueous droplets. This type of system suffers from the need to use organic solvents which would be undesirable for protein delivery since the solvent may denature the protein. Also, these systems often give an initial burst release of the drug. Therefore, it is envisioned that hydrophilic biodegradable network polymers will improve the range of drugs delivered from this general class of polymers.

Biodegradable Nanoparticles

The use of nanoparticles for colloidal drug delivery has been a goal of formulation scientists for the last 20 years. Nanoparticles are defined as any solid particle between 1 and 1000 nm, and are composed of natural or more commonly synthetic polymers. The most useful method of production for the lower end of this size range is emulsion polymerization, where micelles act as a reaction template for the formation of a growing polymer particle. For passive delivery of anticancer agents to tumors, nanometer size particles (50-200 nm) are required. The small size is required for extravasation of the nanoparticles through the

permeable tumor vasculature in a process termed the EPR effect (enhanced permeability and retention) (Duncan).

Another important feature of any nanocarrier is the biocompatibility of the particle. This requires that the polymer particle be non immunogenic an that it degrade after some period so that it may be excreted. These criteria require polymer compositions that are well tolerated.

Hydrogel particles can be made in several sizes according to the performance requirements of the drug delivery system being engineered. Gel particles in the nanometer size range that are capable of being retained in tumor tissue are preferred for delivery of anticancer agents. Methods for the creation of approximately 100 nm in diameter hydrogel particles involve the use of surfactant-based emulsion polymerizations in water. To make ionomeric nanogels by this method it is necessary to include a hydrophobic component in the monomer mixture, thus allowing partitioning of the monomers into the micellar phase followed by particle nucleation and further monomer adsorption (normally emulsion polymerizations are used to make hydrophobic latexes).

Another important consideration is the means by which the carrier will load the drug substance to be delivered. The loading capacity of non-ionic hydrogels is generally limited by the aqueous solubility of the drug. However, if the drug is charged, groups of opposite charge to the drug can be incorporated into the polymer to allow high drug loading through ion exchange. An interesting and perhaps useful property resulting from inclusion of charged monomers in the polymer network is a pH induced volume response of the polymer.

Current State of the Art

To date most biodegradable polymers have been synthesized using stepwise condensation of monomers resulting in a polydispersed molecular architecture. Since the rate of degradation is in part directly related to this architecture, this method results in the undesirable property that the material will contain cross-links with a variety of degradation rates. Secondly, since synthetic biodegradable polymers are generally water insoluble, there is a need for degradable moieties that are readily incorporated into water soluble monomers or polymers. Biodegradable moieties based on the non-soluble degradable units can be combined with water soluble oligomeric regions or polymers, resulting in a biodegradable structure.

Previous work in the area of creating biodegradable cross-linkers by Hubbell teaches a method to create such degradable sequences using ring opening polymerization of lactide or glycolide with polydispersed water soluble groups which are then terminated with a reactive group. This method creates a mixture of polydispersed degradable units with varying molecular weights or chain lengths in the end product. Since the length of the degradable region is and will be shown to be a major structural determinate of the degradation rate, there is a lack of control over the rate of gel degradation. This is because the degradable regions with more lactic or glycolic residues degrade faster, for there are more cleavage sites, and only one ester link must cleave for the cross-linker and thus gel to degrade.

The present invention described herein teaches a method of stepwise synthesis of the degradable region which creates a pure, monodispersed compound at the end of the synthesis. Since the length of the degradable region will be the major structural determinant

of the degradation rate, the present invention provides for a more controlled degradation rate than the Hubbel invention.

Therefore, as an object of the present invention a new more desirable cross-linker would have the preferred characteristics that it was easily synthesized, composed of biocompatible components, and have a well defined molecular structure leading to defined biodegradation rates. Additionally, the inventive cross-linker should be able to cross-link polymer filaments obtained from a wide variety of monomers and already formed polymers. Such variety would include monomers and/or polymers that are neutral, charged, hydrophilic, hydrophobic, synthetic, and natural. Further, it is an object of the present invention that the inventive cross-linker be easily incorporated in many different polymer processing options such as polymer nanoparticles, microparticles, bulk gels, slab gels, and films of cross-linked networks of polymer filaments.

Therefore, organic synthesis methodology is used to incorporate monodispersed degradable sequences into the cross-linker monomer structure before polymer formation. Since each sequence has differing rates of cleavage of degradation, the invention here permits control of overall degradation as well as the release rate of entrapped substances and other uses.

Our invention also provides compounds which will be easier to purify than the Hubbell invention owing to stepwise syntheses of the degradable region and the resulting purity of the reaction product. Other advantages of our invention over Hubbell's invention are that the invention described herein is applicable to hydrophobic networks as well as hydrophilic networks whereas Hubbell is restricted to hydrophilic networks, and the invention herein can generate all useful properties such as rapid degradation rate and water

solubility through the syntheses of oligomeric cross-linking compounds without resorting to polymeric cross-linking compounds.

SUMMARY OF THE INVENTION

In one important aspect the present invention concerns a monomeric or oligomeric cross-linker comprising a monodispersed polyacid core with at least two acidic groups directly or indirectly connected to a reactive group usable to cross-link polymer filaments or other substances. Such cross-linkers also have at least one acidic group of the polyacid core directly or indirectly connected to a monodispersed region degradable under aqueous or environmental conditions and where these degradable regions or (in the case of a single degradable region), the degradable region have at least one other acidic group covalently attached directly or indirectly to a reactive group usable to cross-link polymer filaments. Thus, at least two reactive groups are always interspersed by at least one degradable region.

In many preferred applications, the cross-linker is utilized to cross-link water soluble polymeric filaments. The polyacid core may be attached to a water soluble region that is in turn attached to a degradable region having an attached reactive group. Alternatively, the polyacid core may be attached to a degradable region that is in tern attached to a water-soluble region having an attached reactive group. A polycarboxylic acid is the preferred polyacid. The polyacid core is preferably a diacid, triacid, tetraacid or pentaacid. The most preferred polyacid core is a diacid. Preferred polyacids or polycarboxylic acids include alkyl-based diacids such as oxalic, malonic, succinic, glutaric, adipic, fumaric, maleic, sebacic and tartaric are preferred. Diacids such as succinic, adipic or malonic acid are particularly preferred. A triacid such as citric acid, for example, may also be used. Tetra-and penta-acids such as ethylenediamine tetraacetic acid (EDTA) or diethylenetramine pentaacetic acid (DTPA), for example, are usable. When cross-linked polymer filaments are formed according to the present invention, they are cross-linked by a component having at least one degradable region. Preferred degradable regions include ester linkages and

especially those esters linkages of mono or poly (alpha-hydroxy acids), although other hydroxy alkyl acids that may form polyesters can be used to form biodegradable regions. Preferred esters and polyesters include those of glycolic acid, DL-lactic acid, L-lactic acid, oligomers, monomers or combinations thereof. Cross-linkers of the present invention may also include a degradable region containing one or more groups such as an anhydride, an orthoester, an acetal, a ketal, a sulphonic ester, a carbonate, a phosphoester, or other groups biodegradable groups known to those in the art. In certain cases the biodegradable region may contain at least one amide functionality. The cross-linker of the present invention may also include a water soluble region such as tetraethylene glycol, a diethylene glycol, an ethylene glycol oligomer, oligo(ethylene glycol), poly(ethylene oxide), poly(vinyl pyrolidone), poly(propylene oxide), poly(ethyloxazoline), or combinations of these substances.

Preferred reactive groups are those that contain a carbon-carbon double bond, a carbonate, a mixed anhydride, a carbamate, a hydrazone, a hydrazino, a hydrazide, a cyclic ether, an acid halide, a acylazide, a succinimidyl ester, a maleimide, a thiol, a disulfide, an imidazolide or an amino functionality. Other reactive groups may be used that are known to those skilled in the art to be precursors to polymers or capable of grafting to polymers.

One preferred embodiment of the present invention is as cross-linkers which are composed of a symmetrical diacid attached to at least one biodegradable region. These regions may consist of alpha hydroxy acids e.g., glycolic or lactic acid. These degradable portions are then terminated directly or indirectly by a functional group which may be polymerized. Moreover, cross-linker component pieces of the degradable gel such as lactic, and succinic acids are members of the Krebs cycle and therefore are readily metabolized

in vivo, while the end groups become incorporated into water-soluble polymer, which is eliminated by renal excretion.

Utilizing the cross-linkers of the present invention, networks of polymer filaments may be formed by thermal, catalytic or photochemical initiation. Also, polymerization may be effected by plasma or ionizing radiation. Networks of polymer filaments may likewise be formed by pH changes. Networks of polymer filaments may also be formed, for example, by free radical addition, Michael addition or condensation.

The present invention also comprises a network of polymer filaments formed by precipitation, suspension, emulsion, or other polymerization methods and cross-linked by a monomeric or oligomeric cross-linker comprising a monodispersed polyacid core with at least one acidic group connected to or as part of a monodispersed region degradable under physiological conditions and having at least two covalently attached reactive groups usable to cross-link polymer filaments. Polymeric filaments to be cross-linked include preformed polymer filaments such as polynucleic acids, polypeptides, proteins or carbohydrates. Such cross-linked polymeric filaments may also contain biologically active molecules. The biologically active molecules may be organic molecules, inorganic molecules, peptides, proteins, carbohydrates, polynucleic acids, and bioconjugates.

The preferred monomeric or oligomeric cross-linker of the present invention has a monodispersed polyacid core with a molecular weight between about 30 and about 2000 Daltons. The degradable regions have a preferable molecular weight range of about 30 to about 1800 Daltons. The reactive groups of the cross-linker of the present invention may be end groups and have preferred molecular weights between about 10 and 400 Daltons.

An important aspect of the present invention is a monomeric or oligomeric cross-linker comprising a monodispersed polyacid core with at least two esterified groups

being connected (directly or indirectly) to reactive groups usable to cross-link polymer filaments. Between at least one reactive group and polyacid core is a monodispersed region degradable under aqueous conditions. Thus, the cross-linker is usable to form a network of cross-linked polymer filaments. In a preferred embodiment, the polyacid core has two acidic groups connected to a monodispersed region degradable under aqueous or other environmental conditions, each having a covalently attached reactive group usable to form cross-linked polymer filaments. In certain cases the cross-linkers of the present invention may contain a water soluble region located between at least one carboxyl group and its associated reactive group. A preferred polymer filament for cross-linking is a hydrogel. In certain cases the polymer filament being cross-linked may be hydrophobic.

In many cases the polyacid core of the present inventive cross-linker is a diacid, such as oxalic, succinic acid, glutaric, adipic acid, fumaric acid, maleic acid, sebacic acid or malonic acid, for example. Triacids such as citric acid are also usable. Other triacids will be apparent to those of skill in the art. Tetraacids and pentaacids may also be used. A preferred tetraacid is ethylene diamine tetraacetic acid (EDTA) and a preferred pentaacid is diethylenetriamine pentaacetic acid (DTPA). Acids that may be used as a monodispersed polyacid core include citric acid, tartaric acid and the like.

A preferred biodegradable region for use in the cross-linkers of the present invention is one that comprises an ester--especially of a hydroxy alkyl acid. A preferred hydroxy acid ester is an alpha hydroxy acid ester. Under some circumstances the degradable region may be a peptide. Preferred degradable polyesters include glycolic acid polyester, DL-lactic acid polyester and L-lactic acid ester or combinations thereof. In certain cases the degradable region of the cross-linker of the present invention may comprise anhydride, orthoester, sulphonic acid ester or phosphoester linkages. A reactive group is capable of forming

polymers or attaching to polymers. In certain cases the reactive group of the present inventive cross-linker contains a carbon-carbon double bond. In some cases the reactive group is an end group, e.g. at the end of a degradable region. The reactive group may also contain a carbonate, carbamate hydrazone, hydrazino, cyclic ether, acid halide, acyl azide, alked sulfonate, succinimidyl ester, imidazolide or amino functionality.

The cross-linker of the present invention may be utilized to form networks of polymer films formed by thermal, catalytic, or photochemical initiation. In certain cases networks of polymer films may be formed as induced by a pH change and then cross-linked. In other cases, networks of polymer films may be formed through reactions involving free radical addition or Michael addition. The aqueous conditions under which the cross-linkers of the present invention are degradable are most frequently physiological conditions.

In an important aspect, the present invention comprises a network of cross-linked polymer filaments formed by precipitation, dispersion or emulsion polymerization and cross-linked by a monomeric or oligomeric cross-linker having a monodispersed polyacid core with at least two esterified groups connected to a covalently attached reactive group used to cross-link polymer filaments and at least one acidic group having a monodispersed region degradable under aqueous conditions between the acidic group and the reactive group. This network may be formed or shaped into rods, fibers, and other geometric shapes.

Also included in the present invention are networks of polymer filaments of polynucleic acids, polypeptides, proteins or carbohydrates and cross-linked by a monomeric or oligomeric cross-linker comprising a monodispersed polyacid core with at least one esterified group connected to at least one region degradable under physiological conditions, and having a covalently attached reactive group cross-linking the polymer filaments.

In both cases of a network of polymer filaments, these networks may contain biologically active molecules. Because the cross-links are degradable, these biological molecules will be expected to be released as the network degrades.

In one important aspect, the present invention comprises a network of polymer filaments cross-linked by a monomeric or oligomeric cross-linker comprising a monodispersed polyacid core with at least two acidic groups connected to at least one region degradable under physiological conditions, and both acidic groups connected to a covalently attached reactive group. Such a network may be defined further as comprising an organic molecule, inorganic molecule, protein, carbohydrate, poly(nucleic acid), cell, tissue or tissue aggregate.

For environmental purposes, products of cross-linked polymer networks, particularly after usage and discard, the degradable groups should be biodegradable under existing environmental conditions.

Additionally, the invention includes a network of polymer filaments cross-linked by monomeric or oligomeric cross-linker comprising a central polyacid core with at least two acidic groups connected to at least one region degradable under physiological conditions, and terminated by a covalently attached reactive end group usable to cross-link polymer filaments, the network comprising an organic radioisotope, inorganic radioisotope or nuclear magnetic resonance relaxation reagent.

According to the present invention the polyacid core has a preferred molecular weight between about 30 and about 2000 daltons. The degradable region of the cross-linker has a preferred molecular weight between about 30 and about 1800 daltons. The reactive groups of the present invention generally have molecular weights between about 10 and about 300 daltons.

Use of more the one cross-linking monomer in a single product

Besides the use of a single degradable cross-linker of the present invention to cross-link polymer filaments, applications where more than one type of cross-linker may be useful are envisioned. The use of a known nondegradable cross-linker (such as divinyl benzene or methylenebisacrylamide) in combination with a degradable one of the present invention may be useful. The resulting polymer network would degrade until only the non degradable cross-links remained. Such a product may allow much wider pores to be engineered into a product, allow greater absorbency to be obtained, or allow recovery of a gel after releasing its contents (e.g. drug, protein, or other active ingredient) upon cleavage of the degradable links. Uses of more than one degradable cross linker of the present invention is also envisioned. For example, if two inventive cross-linkers with very different degradation rates were used to make a controlled release device or particle of cross-linked polymer filaments, the resulting product would be expected to have two different degradation or release profiles. Thus, a burst release of drug or other substance could be engineered into such a delivery device. Alternatively, the use of two or more cross-linkers with different degradation rates of the present invention may be used to produce a network of cross-linked polymer filaments. Such a network may have a degradation rate intermediate between the degradation rates of the networks composed of a single inventive cross-linker. Of course, a degradable cross-linking monomer already known to those skilled in the art may be useful in combination with the present invention.

Range of molecular weights

The molecular weight ranges for the present invention are chosen to include from small to very large monodispersed polyacids and up to twelve degradable units (e.g.

hydroxy acid esters). The smallest acid or part thereof is a simple carbonyl with molecular weight 30. One of the largest polyacids envisioned for use as the polyacid core is a substituted gamma cyclodextrin. Since the cyclodextrin itself has a molecular weight of 1280 Da, ones substituted with acids and other groups may range up to 2000 Da. Accordingly, the molecular weight range for the polyacid core of the present invention is 30 - 2000 Da. For the degradable region, the smallest one is also a carbonyl of an ester from example. At the other extreme one of the largest degradable units is an ester of mandelic acid (152 Da). Since each degradable region may contain up to six such units and the inventive cross-linkers often have two such regions the upper size for degradable regions is about 1800 Da. The molecular weight ranges for the reactive groups is 10 to 400. This range includes an open position on a boron atom to a penta-allyl ether of a hexose with a molecular weight of about 400 Da.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 schematically illustrates representative lactate or glycolate-based cross-linking agent of the present invention.

FIG. 2 shows the synthetic schemes for symmetrical biodegradable cross-linkers such as HPMALacSuc **5a**, HPMAGlySuc **5b**, HPMALacLacSuc **7a**, and HPMAGlyGlySuc **7b**. Conditions: (a) CH2Cl2, pyridine 0 °C; (b) Pd/C 50 psi H2, i-PrOH; 0 °C; (c) carbonyldiimidazole (CDI), DMF, 0 °C; HPMA, rt.; (d) CDI, DMF, 0 °C; (S)- benzyl lactate or benzyl glycolate (e) Pd/C 50 psi H2, i-PrOH.

FIG. 3 displays a photograph of biodegradable gels of the same composition with 1.5 mole % cross-linker after incubation in pH 7 phosphate buffer at 37°C for varying amounts of time. **(a)** control gel made up of compound **2** and HPMA after 15 days. **(b-d)** degradable gel made up of compound **5b** and HPMA after 2, 5 and 15 days, respectively.

FIG. 4 shows four plots of the degradative swelling (Qv) versus time in buffers at four pHs. The gels are HPMA-co-XL gels made using four different cross-linkers of the present invention (**2**, **5a**, **5b**, and **7a**) at 1.5 mol%. FIG. 4A shows the degradative swelling of these gels in pH = 0.73 buffer. FIG. 4B shows the degradative swelling of these gels in pH = 4.0 buffer. FIG. 4C shows the degradative swelling of these gels in pH = 7.3 buffer. FIG. 4D shows the degradative swelling of these gels in pH = 9.0 buffer. All swelling studies were done at 37 °C and I=200 mM.

FIG. 5 is a plot of the half-life to dissolution versus pH for HPMA-co-XL gels made from 3 different degradable cross-linkers studied at 37°C for a range of pHs.

FIG. 6 displays a photograph of poly(HPMA) degradable gels with 1.5 mole % cross-linker and containing a deep red fluorescent dye, thus the dark color, after incubation in pH 7 phosphate buffer at 37°C for varying amounts of time. **(a)** control gel made from

compound 2 after 15 days; (b and c) gels made from compound 7b after 4 and 8 days respectively.

FIG. 7 displays a plot comparing the swelling response and the release of tetramethyl rhodamine labeled albumin from the degradable gel network for HPMAGlyGlySuc 7b at pH 7.3 buffer at 37°C.

FIG. 8 shows the synthetic schemes for symetrical cross-linker with nonvinyl reactive groups such as HydLacSuc 8a, HydGlySuc 8b, NHSLacSuc 10a, and NHSLacSuc 10b. Also shown is the scheme for asymmetric cross-linkers HPMALacSucHPMA 9a and HPMAGlySucHPMA 9b. Conditions: (a) THF, pyridine isobutyl chloroformate; (b) carbobenzyloxyhydrazide 0 °C; (c) Pd/C 50 psi H2 (d) DCM, pyridine, succinic anhydride, (e) Pd/C 50 psi H2, (f) CDI, DMF; HPMA, (g) disuccimimidyl carbonyl.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions

Water solubility indicates that a compound is soluble to at least to 1 gram per 100 mL of water under ambient conditions. Physiological conditions are those typically encountered in living organisms, especially mammals. Environmental conditions, especially regarding cross-linker degradation, refers to conditions of temperature, pH, and sunlight that may be encountered near the earth's surface. Typically, this means temperatures between -40 °C to 50 °C, aqueous pHs of 1 to 12, and dark or sunny conditions. Hydrophilic refers to monomers that may dissolve in water or cross-linked polymer filaments that swell when placed in aqueous solution. Hydrophobic refers to monomers that are not water soluble and cross-linked polymer networks that do not swell when placed in water. Rather, hydrophobic molecules and networks would dissolve or swell in organic solvent, respectively. A 'network polymer' means a network of cross-linked polymer filaments.

The term 'monodispersed' refers to compounds or regions (i.e polyacid cores or degradable regions, respectively) with a single molecular weight and structure rather than a range of molecular weights or an ambiguous structure. However, cross-linkers that are enantiomeric or diastercomeric are included in the present invention. For example, a degradable region of three glycolic acid residues (GlyGlyGly) is monodispersed, whereas a degradable region with two to four glycolic acid residues (i.e. -GlyGly- or -GlyGlyGly- or -GlyGlyGlyGly-) is polydispersed.

Description

This invention discloses representative syntheses and applications of symmetrical and asymmetrical biodegradable cross-linking agents for use in cross-linked polymer matrices formed into particles, films, or slabs that may be used *e.g.*, in drug delivery. The

cross-linking agents will be monomers or oligomers of biocompatible units in the preferred biological applications. For non biological applications the cross-linking agents do not necessarily need to be of biocompatible units. In the preferred practice of this invention the cross-linker is composed of a central diacid (such as succinic); to this diacid is attached one or more biodegradable regions, which are then terminated by reactive moieties which are used for incorporation into the polymer network. This invention requires there be at least two reactive moieties (two representative cross-linkers are depicted in FIG. 1). The cross-linkers may be incorporated into matrices of various sizes ranging from hundreds of cm's to less than a nanometer, so as to control the diffusion of substance such as drugs *e.g.*, from the matrix by biodegradation of the cross-linkers under physiological conditions. Ultimately the cross-linkers described above may be included in all variety of hydrophilic and hydrophobic polymer networks to which the desirable property of degradation is required.

Design of degradable cross-linkers based on the alpha-hydroxy acids

Of importance in hydrogel engineering is the control of the structural properties of a random network of cross-linked polymer filament. In standard stepwise growth of polymers there is heterogeneity in copolymer composition and dispersity in the molecular weight of the polymer filaments thus making it difficult to precisely control bulk material properties of the polymer network such as crystallinity and mesh size. By engineering homogeneous or monodispersed polyacid cores and degradable regions into the polymer structure or matrix, usefully tuned macromolecular properties such as biodegradability or environmental degradability can be obtained.

Hydrogel networks in the form of colloidal particles which are being explored for use in drug delivery (Kiser *et al.*) are not biodegradable owing to their carbon-carbon bond containing backbone and their non degradable methylenebisacrylamide cross-links. This

fact initiated the design of a new class of symmetric and asymmetric cross-linking monomers. One of the preferred characteristics of the new material was that it must be easily synthesized. A second preferred characteristic is that the cross-linkers be composed of biocompatible components. The third characteristic which separates this work from all other work in this area is that the biodegradable cross-linker may be synthesized to be monodispersed in the polyacid core and degradable region(s) being a single pure molecule and not a mixture. This characteristic should lead to defined biodegradation rates versus the use of a cross-linker mixture as in previous work (Pathak *et al.*).

Therefore, the utilization of classical organic synthesis methodologies to synthesize monodispersed degradable sequences into the monomer structure before polymer formation presents an opportunity to carefully control the overall degradation as well as possibly the release rate of entrained substances. One of the particularly preferred embodiments of these cross-linkers is that they are composed of a symmetrical diacid with each acid group attached to a biodegradable region consisting of acids, such as the alpha-hydroxy acids glycolic or lactic acid, for example. These portions are then preferably covalently attached or terminated to a methacrylate or methacrylamide group.

The Cross-linkers

The cross-linkers are composed of a central polyacid as shown in FIG. 1 and are attached to the degradable region through oxygen, nitrogen, sulfur, or phosphorous atoms. Structure A shows a cross-linker having a central diacid region (———), and a degradable region (WWWW) which is then terminated by a reactive polymerizable region (- - - - - - -X). Structure B is similar and uses the same symbols except that the central core is a triacid symbolized by a T structure. Structures C and D display a more specific embodiments of this invention. In structure C, a symmetrical centerpiece (succinic acid, n=2) is attached to

one or more degradable regions containing alpha-hydroxy esters (a = 0,1,2...6, b = 0, 1, 2...6). These are then attached to a moiety (R_2) which may or may not impart water solubility through the connecting portion labeled Y. Finally, the cross-linker is terminated with vinyl groups. Structure D is again similar to structure C except in this case the monomer is terminated with two nucleophilic moieties which could be used to cross-link preformed polymer chains. These structures are exemplary only. Many more are conceivable by those skilled in the art.

In a preferred embodiment the network begins with a cross-linker containing two equal degradable regions attached to a central diacid and each containing a terminal reactive group. In a particularly preferred embodiment, the core is made of succinic acid, each degradable region is composed of either symmetrical units of glycolic or lactic acid where in FIG. 1 is between 1 and 5 and the terminal reactive group is a acrylate type moiety where R2 in FIG. 1 is Y-CH(CH3)CH_2C(O)- and Y is equal to oxygen.

Central Component

In preferred embodiments the central piece can consist of esters of dicarboxylic acids such as oxalic, malonic, succinic, glutaric, adipic, sebacic, maleic, and fumaric acids or even possibly (alpha, omega-(oligo(ethylene glycol))dicarboxylic acid, and (alpha, omega-(oligo(propylene glycol)) dicarboxylic acid as long as they are monodispersed. Other diacids such as aromatic polycarboxylic and polysulphonic acids may also be used. In another embodiment tri-acids such as citric acid or tetra and penta acids such as EDTA and DTPA (possibly as protected derivatives) could also be utilized. Also, hydroxyl protected versions of tartaric, citric acids as well as amino protected versions of aspartic or glutamic acid may be used in certain embodiments.

Biodegradable Component

The biodegradable region is preferred to be hydrolyzable under environmental or *in vivo* conditions. In the most preferred embodiment the degradable regions will be composed of glycolic or lactic acid domains containing anywhere from one to six members in each oligomeric region attached to the central piece. Other hydroxy esters that may be embodied include: 3-hydroxy butyric acid, 2-hydroxypropanoic acid, mandelic acid, and 5-hydroxycaproic acid. Other useful biodegradable regions include amino acids, ortho-esters, anhydrides, phosphazines, phosphoesters and their oligomers and polymers as long as they are prepared in a monodispersed fashion.

Reactive Cross-linking Polymerizable Region

This region is necessary for the invention because it is the chemical functionality terminating or juxtaposing the two or more ends of the cross-linker which will chemically bind polymer filaments together. A preferred method of achieving this end is through an acrylate moiety, with polymerization through free radical generation. Free radical generation can be accomplished via thermal, photochemical, or redox catalysis initiation systems (Odian). Also, free radical generation may be by plasma or ionizing radiation. The preferred polymerizable regions for free radical generation are acrylates, vinyl ethers, allyl ethers, diacrylates, oligoacrylates, methacrylates, dimethacrylates, and oligomethacrylates. Alternatively another preferred method of cross-linking preformed chains in solution is to attach one or more nucleophilic or electrophilic groups to the end, or ends, of the chains which would be reactive with its complementary type attached to a formed or forming polymer chain. Preferred chemical reactive moieties for this method are carbonate, carbamate, hydrazone, hydrazino, hydrazide, cyclic ether, acid halide, acyl azide, alkylazide, succinimidyl ester, imidazolide, maleimide, amino groups, alcohol, carbonyl, carboxylic

acid, carboxylic ester, alkyl halide, aziridino, nitrile, isocyanate, isothiocyanate, phosphine, phosphonodihalide, thiol, sulfide, disulfide, sulfonate, sulfonamide, sulfate, silane, silyloxy groups, or other such groups known to those in the art.

Initiators

Several initiation systems for the formation of polymer networks are useful with these compounds, depending on the application and the conditions used.

For generation of polymer slabs either irradiation of vinyl groups with high energy light such as in the UV is a suitable method for initiation. Other preferred methods include the use of thermally activated initiators such as azobisisobutyronitrile or benzoyl peroxide for initiation in water or mixed water/organic solvents, other water soluble alkyl diazo compounds, ammonium persulphate with or without N,N,N',N'-tetramethyethylene diamine. Also, plasma and ionizing radiation may be used for generation of particles by emulsion polymerization generation of radicals by thermal initiation is convenient. Generally this is accomplished with water soluble initiators such as ammonium persulphate. Other initiators include the water soluble alkyl diazo compounds.

For generations of polymer networks *in vivo* the most useful initiation system is photochemical. Photochemical initiation of free radical polymerization involves light activation of a light absorbing compound (a dye), radical abstraction of a hydrogen to generate the initiation radical (usually an amine), and attack of this radical on a vinylic moiety beginning the polymerization. This system preferably requires free radicals to be generated locally and within a short time period, preferably in seconds. Initiation in this system begins with irradiation of light at the appropriate wavelength. The wavelength is chosen to be as close to the absorption maximum of the dye as possible. The preferred light absorbing compounds which will begin the radical generation process are eosin dyes, 2,2'-

dimethoxy-2-phenylacetophenone and other acetophenone derivatives. Other photo redox active dyes include acridine dyes, xanthene dyes and phenazine dyes, for example, acriblarine, rose bengal and methylene blue, respectively. These dyes when photoactivated assume a triplet excited state which can abstract a proton from an amine and thus generate a radical which begins the polymerization. Compounds which act as the initiating radical are amines such as triethanolamine, sulfur containing compounds such as ammonium persulphate, and nitrogen containing–heterocycles such as imidazoles.

Applications for the cross-linkers

Nature of the polymer

In the preferred embodiment of this invention, these cross-linkers can be incorporated in biodegradable network polymers that are either hydrophilic or are hydrophobic. Hydrophobic networks will contain less than 5% of the total mass of the polymer network as water. Whereas hydrophilic networks can contain as great as 99% water as the total mass. Hydrophilic network polymers are known as hydrogels to those skilled in the art. Those skilled in the art will generally recognize the polymer structures which are generally considered to be hydrophilic or hydrophobic. Typically, hydrophilic network polymers swell with water.

***In Vivo* Drug Delivery**

One preferred application of these materials is in the use of controlled delivery of bioactive compounds. In this method the cross-linker is homopolymerized or copolymerized with other monomer or polymers which may be charged or uncharged and hydrophillic, or hydrophobic. The drug is placed in the polymer network by polymerizing the network around the drug (*i.e.*, by co-dissolving or dispersing the drug with the monomer

solution) or by incubating the resulting polymer with a solution of the drug whereby it diffuses into the polymer network. In this embodiment the drug may be anywhere from 0.1 to 90% by weight of the device. The biologically active compounds can be (but are not limited to) proteins, peptides, carbohydrates, polysaccharides, antineoplastic agents, water soluble linear and branched polymeric prodrugs, particles containing drug, antibiotics, antibodies, neurotransmitters, psychoactive substances, local anesthetics, anti-inflammatory agents, spermicidal agents, imaging agents, phototherapeutic agents, DNA, oligonucleotides, and anti-sense oligonucleotides.

An alternative method of producing a biodegradable drug delivery system is through the production of particles. The preferred size range is between 1 nm and 10 μm. These particles can be produced by emulsion polymerization in water containing a surfactant such as sodium dodecyl sulfate, an initiator such as ammonium persulphate, and cross-linking monomer and co-monomer(s) such as 2-hydroxypropylmethacrylamide, 2-hydroxyethylmethacrylate, acrylic acid, methacrylic acid, methyl methacrylate, methyl acrylate, or other suitable monomers by themselves or in mixtures. Alternatively the particles can be synthesized by precipitation polymerization in organic solvent containing organic soluble initiator such as azobisisobutyronitrile and co-monomer(s) such as acrylamide, as 2-hydroxypropyl methacrylamide, 2-hydroxyethyl methacrylate, acrylic acid, methacrylic acid, methyl methacrylate or methyl acrylate by themselves or in mixtures. In this method the preferred route of incorporating drug in the particles is by first synthesizing the particle, followed by purification through washing. The particle is then incubated with drug which is then bound or emeshed within the polymer network by either hydrophilic, hydrophobis, or ionic forces or by entrapment within the network.

Another method which is well known to those skilled in the art of producing polymer particles includes dissolving the cross-linking monomer, co-monomer, initiator with or with out the drug in water and then dispersing this solution in oil. The resulting oil droplets then act as templates for the formation of the gel network. Polymerization is initiated either thermally, chemically or photochemically depending on the monomer system and initiator system. Which combination of systems to use will be obvious to those skilled in the art. The resulting particles can then be sedimented and isolated and purified. This technique is particularly useful for producing larger particles in the 5- to 1000 micron in diameter size range.

Another preferred method for the creation of a drug delivery device is to create a homopolymer network of the cross-linker in organic solvent in the presence of an organic soluble drug. The network is then dried and contains drug dispersed within it. The highly cross-linked network will begin to erode when hydrated and release drug.

Water Absorbents

In this application an important consideration is to copolymerize the biodegradable cross-linker with charged monomers (either negative charges or positive charges or mixtures thereof). Very high charge densities within the polymer network can be obtained by copolymerization of charged monomers into networks (>5 M charges). The presence of charges in the polymer network require counterions for electroneutrality. These counterions bind water to a lesser or greater extent, depending on their charges, size, and polarizabilities. Since the volume of the hydrated gel is equal to the volume of polymer, the volume of water bound to the polymer and the volume of the hydrated ions bound to the polymer, the presence of a large amount of hydrated ions can create a super-water adsorbent hydrogel. The molar ratio of cross-linker to other monomers should be kept as low as

possible so as to not inhibit the swellability of the network, preferably in the range of 5 mol % or less. The preferred copolymers include methacrylic acid, acrylic acid, acrylic and methacrylic monomers containing sulfate, alkyl carboxylate, phosphate, amino, quaternary amino and other charged groups and their salts. In this application large batches of the degradable network will be synthesized either by dispersion polymerization or in bulk. The material could be synthesized in the presence of a suitable counterion such as sodium for negatively charged filaments or chloride for positively charged filaments. Alternatively the polymer may be formed in its neutral state and then incubated with a suitable acid or base such as hydrochloride in the case of nitrogen containing co-monomers, and soluble metal hydroxides in the case of acidic co-monomers. The most preferred method (Buchholz) is to polymerize the cross-linker with a mixture of the salt form and the acid form of the co-monomer(s).

Such degradable absorbant and superabsorbant hydrogels may be used in products for which water or liquid absorption is an important component of operation, for example diapers, sanitary products and wound dressings (Tsubakimoto et. al, Brandt et. al). The primary advantages of using hydrogels of the present invention as absorbants or superabsorbants in such products are a) their capacity for the liquid increases as the cross-linker degrades, and b) the hydrogel can degrade completely to water soluble components following disposal, so lessening the negative environmental impact caused by the disposal of such items.

Adhesives

Another use of the cross-linking monomer is in temporarily binding two surfaces together. The biodegradable cross-linking monomer and co-monomer or just the biodegradable cross-linking monomer itself are mixed together with a solvent and an initiator by itself or with a co-catalyst. The mixture is then spread on the surfaces which are to be adhered, then polymerization is initiated by addition of heat or by light. In the case of light initiation at least one of the surfaces to be adhered must be transparent to the light beam in order for the polymer network to form. The initiation systems described above can be used to this end. Such biodegradable adhesives should have many uses.

Tissue Supports

There is a need for degradable polymers as cell scaffolds in tissue engineering (Langer). In this application the tissue scaffold would be synthesized under sterile conditions in a suitable biocompatible buffer. The cross-linking density should be controlled so as to obtain a pore size large enough to allow cell migration or degrade as the cells migrate and grow. Pore size may be determined by scanning electron microscopy and by using macromolecular probes. A cell suspension containing cells such as, but not limited to, keratinocytes, chrondocytes and osteoblasts, would be injected into the polymer network along with suitable growth factors. The cells would then be allowed to grow within the network. As the cells grow the network around them would degrade. Bioadhesive moieties such as RGD peptide sequence (Arg-Gly-Asp) could be connected to matrix and thereby provide adhesive domains for the growing cells. The timing of the network degradation should coincide with the cells forming their own network (artificial tissue) through inter-cell contacts.

Examples

The following examples are presented to describe preferred embodiments and utilities of this invention but are not intended to limit the use or scope of the methods, compositions or compounds claimed in this invention unless otherwise stated in the claims. Taken together, these examples describe the best currently understood mode of synthesizing and incorporating these materials into polymer networks.

The syntheses of the eleven members of the preferred class of molecules claimed herein are given in FIG. 2 and FIG. 8. This invention has several advantages over related inventions in this area, including: (1) the cross-linking agents are biodegradable to biocompatible substances, (2) the syntheses are both general and flexible, allowing for a variety of specific monomeric units to be incorporated, (3) the end groups (*e.g.*, acrylate or hydrazide) can be readily modified to accommodate either condensation or radical-type polymerizations, or other types of polymerizations, (4) the rate of degradation of the polymer filament network made from the inventive cross-linking monomers can be increased or decreased by altering the number and types of degradable regions (especially those of ester groups), and (5) because degradation takes place at a defined rate due to the monodispersed nature of the degradable region of the inventive cross-linking monomers, no burst release of entrained molecules or other species is expected to occur.

Materials and characterization

All chemicals were reagent grade and were used without purification unless otherwise noted. 1H NMR and 13C NMR spectra were recorded at 400 and 100.4 MHz respectively on a Varian INOVA-400 spectrometer equipped with a temperature-controlled probe. Abbreviations for NMR data are as follows: s=singlet, d=doublet, m=multiplet, dd=doublet of doublets, t=triplet. Melting points are uncorrected. Coupling constants (*J*)

are reported in Hertz. Chemical shifts are reported in parts per million. 1H shifts are referenced to CHCl3 (7.24) or to DMSO (2.54), and 13C spectra are referenced to CHCl3 (77.14) or to DMSO (40.45). Solvent mixtures are given in volume to volume ratios unless otherwise stated. Flash chromatography was performed on SiO2 Kieselgel 60 (70-230 mesh E. Merck). Mass spectroscopy was performed at the Duke University Mass Spectrometry Laboratory. Optical rotations were obtained using the Na+ 589 nm line at in CHCl3 or acetone using a Perkin-Elmer 241 polarimeter in a 1dm cell. TLC was performed with Whatman AL SIL G/UV plates.

THF was used freshly distilled from sodium benzophenone ketyl under nitrogen. 2-propanol was dried by distilling from CaO and storing over 4A molecular sieves. Dichloromethane was distilled from P205 and stored over molecular sieves. All other solvents were obtained in their anhydrous state or stored over molecular sieves before use. Unless otherwise indicated hydrogenations were performed on Parr hydrogenator at 30 to 50 psi of hydrogen gas.

In the shortened nomenclature used here, 'Bn' indicates a benzyl group, 'Lac' indicates a lactic acid group (usually as an ester of (S)-lactate), Gly indicates a glycolic acid group (usually as an ester), 'Suc' indicates a succinoyl group, 'HPMA' indicates 2-hydroxylpropyl methyacrylamide (usually racemic), 'HEMA' indicates hydroxylethylmethacrylate, 'Adp' indicates the adipoly group, 'Hyd' indicates a hydrazine moiety, and 'NHS' indicates a N-hydroxysuccinimide group.

The nomenclature for symmetrical cross-linkers indicates only one half of the cross-linker. Thus, HPMALacLacSucLacLacHPMA is called HPMALacLacSuc. Similarly, HPMAGlySucGlyHPMA is more simply referred to as HPMAGlySuc. For asymmetric cross-linkers the full sequence is indicated. Thus, **9b** is HPMAGlySucHPMA.

Example 1. Synthesis of symmetrical biodegradable cross-linker (HPMALacSuc) (5a)

Preparation of di(S)-1-[benzyloxycarbonyl]ethyl butane-1,4-dioate (BnLacSuc) (3a). Compound 3a was prepared by reaction of benzyl (S)-(-) lactate (27.0 g, 150 mmol) with pyridine (15.2 mL, 188 mmol), and succinyl chloride (8.21 mL, 75.0 mmol) in dichloromethane (100 mL) at 0°C with subsequent stirring for 16 hours at 25°C. An additional aliquot of succinyl chloride (1.6 mL, 15 mmol) was then added to ensure complete consumption of benzyl lactate. The reaction was allowed to stir 4 additional hours. After filtering the suspension through activated carbon, the dark solution was washed with 100 mL water, 2-50 mL portions of 1M HCl, 2-50 mL portions of water, 2-100 mL portions of sat. NaHCO3 and 100 mL brine. The organic phase was then dried over Na2SO4 and concentrated *in vacuo* to a viscous brown oil. Yield of 3a: 32.3 g (97%). [∝]D = -43.2 (c = 1.0, CHCl3). Elution through a short column (8.5 cm i.d. by 4 cm) of silica gel (70-230 mesh) using 3:7 ethyl acetate/hexane resulted in a yellow oil of high purity by NMR. 1H NMR (CDCl3): 1.49 (d, 6H, J = 7.1 Hz), 2.65-2.72 (m, 4H), 5.08-5.21 (m, 6H), 7.29-7.34 (m, 10H). 13C NMR (CDCl3): 16.63, 28.47, 66.76, 68.68, 76.49, 77.52, 127.91, 128.21, 128.40, 135.13, 170.30, 171.34. Anal. Calcd. for C24H26O8: C, 65.15; H, 5.92. found: C, 65.06; H, 6.02.

Preparation of (2S)-2-{3-[((1S)-1-carboxyethyl)oxycarbonyl] propanoyloxy}propanoic acid (HOLacSuc) (4a). HOLacSuc was prepared by hydrogenolysis of BnLacSuc (3a) (10.2 g, 23.1 mmol) over Pd/C (1.0 g, 10% wt. Pd, Degussa type) in 2-propanol (100 mL). The material was placed on a Parr hydrogenator at 50 psi. at 25°C. When hydrogen uptake had ceased, the sample was removed from the hydrogenator, and the Pd-C was then removed by filtration through celite. The solvent was

removed *in vacuo* at 40°C (16 hours). The crude product was purified by crystallization of its dicyclohexylamine salt as follows: crude **4a** (6.4 g, 23 mmol) was dissolved in 50 mL of a toluene/ethyl acetate/ethanol (2:2:1) solvent mixture. Dicyclohexylamine (9.2 mL, 46 mmol) was added to the diacid solution at 0°C. Crystallization was induced by cooling to -10°C and scratching the sides of the flask. The white solid was washed with 30 mL portions of ethyl ether. Concentration of the mother liquor allowed isolation of a second crop. The first and second crop were combined to give a total yield of 30.2 g [∝]D = -26.9, (c=1.0, CHCl3). The dicyclohexylamine salt was dissolved in 5:1 water/ethanol (10 mL) and subjected to strong cation exchange chromatography (BioRad AG 5OW-X4, 200-400 mesh H form) to regenerate the dicarboxylic acid form. The eluate was lyophilized to remove water/ethanol. The light yellow oil which resulted was taken up in 100 mL dichloromethane/ethyl acetate (5:1) and dried over Na2SO4, to remove residual water. The organic solvents were removed *in vacuo*, and heating the viscous residue to 65°C under vacuum (0.5 mm Hg) was required to induce crystallization of the diacid **4a**. Yield of **4a**: 3.75 g (63%): mp 59-61°C; [∝]D = -54.5, (c = 1.0, CHCl3): 1H NMR (CDCl3): 1.54 (d, 6H, J = 7.1 Hz), 2.72-2.77 (m, 4H), 5.13 (q, 2H, J = 7.1 Hz), 10.97 (br, 2H). 13C NMR (CDCl3): 16.56, 28.51, 68.40, 171.62, 176.28. Anal. Calcd. for C10H14O8: C, 45.81; H, 5.38. found: C, 46.01; H, 5.55.

Preparation of di(1S)-1-{[1-methyl-2-(2-methylprop-2-enoylamino) ethyl]oxycarbonyl}ethyl butane-1,4-dioate (HPMALacSuc) (5a). LacSuc (**4a**) (2.20 g, 8.3 mmol) was dissolved in dichloromethane (30 mL) and cooled to 0°C under an argon atmosphere in a three-necked flask equipped with a stir bar and a powder addition funnel. The reaction vessel was then charged with CDI (2.75 g, 17.0 mmol) via the powder addition funnel. Upon addition of the CDI the reaction frothed copiously. The reaction vessel was

allowed to warm to 25°C, and then HPMA (2.57 g, 17.0 mmol) was added. The reaction was stirred at 25°C for 2 hours, and then washed with 1 M NaH2PO4 (2-100 mL), sat. Na2CO3, (10 mL) and brine (10 mL). The dichloromethane phase was then dried over Na2SO4 and concentrated *in vacuo* to a light yellow, viscous oil. Yield of **5a**: 4.08 g (95%). Although the purity was >90% by TLC and NMR, the purity could be improved by flash chromatography. Elution on 300 mL silica gel (230-400 mesh) using 3% methanol/dichloromethane resulted in 3.22 g (75%) of **5a**: [∝]D= -21.3, (c = 1.0 CHCl3) 1H NMR (CDCl3): 1.24-1.29 (m, 6H), 1.47-1.51 (m, 6H), 1.96 (s, 6H), 2.70-2.74 (m, 4H), 3.20-3.38 (m, 2H), 3.57-3.72 (m, 2H), 4.87-5.00 (m, 2H), 5.03-5.16 (m, 2H), 5.33-5.36 (m, 2H), 5.71-5.75 (m, 2H), 6.25-6.55 (m, 2H). 13C NMR (62.9 MHz, DMSO-d6 several peaks exhibited duality which maybe due to hindered rotation or diastereomers): 16.53, 17.21, 17.37, 18.55, 28.20, 42.99, 54.88, 68.74, 70.17, 70.22, 119.11, 139.83, 139.87, 167.68, 167.83, 169.72, 169.89, 171.27, 171.35. HRMS (FAB+) Calc for C24H27N2O10 (M+H) 513.2448, found 513.2418.

Example 2. Synthesis of symmetrical biodegradable cross-linker HPMAGlySuc

Preparation of di[benzyloxycarbonylmethyl] butane-1,4-dioate (BnGlySuc) (3b). Compound **3b** was synthesized by dissolving benzyl glycolate (15.0 g, 90.3 mmol) and pyridine (7.9 mL, 97 mmol) in 150 mL of CH2Cl2 at 0°C and adding succinyl chloride (4.7 mL 43 mmol), via a syringe while stirring under an argon atmosphere. The reaction was allowed to warm to room temperature and stir for 3 hours. After 3 hours, TLC (5:95 methanol/CHCl3 Rf = 0.5) indicated almost complete reaction, and 0.5 mL of succinyl chloride was added. The reaction was allowed to stir for 12 more hours. The reaction was washed with 2-50 mL of saturated NaHCO3 followed by 2-50 mL 1M NaH2PO4 and then 1-50 mL of brine. The organic layer was dried over Na2SO4. The crude brown solid was

concentrated *in vacuo*. The compound was purified using flash chromatography on a 7 cm i.d. by 40 cm bed of SiO2 eluting isocratically with CHCl3. Alternatively, the solid could be purified by recrystallization from (1:1 ethyl acetate/hexane). The pure fractions were combined and concentrated *in vacuo* to yield **3b** as a white solid. Yield of **3b**: 14.6 g (82%). 1H NMR (CDCl3): 2.77 (s, 4H), 4.65 (s, 4H), 5.17 (s, 2H), 7.29-7.34 (m, 10H); 13C NMR (CDCl3): 28.71, 61.01 67.21, 128.65, 135.13, 167.58, 171.51. Anal. Calcd. for C22H18O10: C, 59.73; H, 4.10 found: C, 59.64; H, 4.25.

Preparation of 2-{3-[(carboxymethyl)oxycarbonyl]propanoyloxy} acetic acid (GlySuc) (4b). Compound **4b** was prepared by dissolving **3b** (5.0g, 11.3 mmol) in 2:1 2-propanol/CH2Cl2 (150 mL) in the presence of 500 mg of Pd/C (Degussa type). The reaction mixture was placed on a Parr hydrogenator at 50 psi for 5 hours, at which time uptake of hydrogen gas had stopped. The reaction was filtered through celite to remove the catalyst and the reaction was concentrated *in vacuo* resulting in a white solid. The solid was triturated with diethyl ether and dried further. Attempts to further purify this material through the dicyclohexylamine salt resulted in low yields due to liability of this material in water. However, the NMR of the triturated product displayed no extraneous NMR resonances. Yield of **4b** 2.54 g (96%): 1H NMR (d6-DMSO): 2.62 (s, 4H), 4.44 (s, 4H), 5.74 (m, 4H); 13C NMR (d6-DMSO): 28.40, 60.61, 169.32, 171.30. HRMS (FAB+) calcd. for C8H10O8 (M+H) 233.0376, found 233.0290.

Preparation of di{[1-methyl-2-(2-methylprop-2-enoylamino)ethyl] oxycarbonyl}methyl butane-1,4-dioate (HPMAGlySuc) (5b). The cross-linker HPMAGlySuc was prepared by adding **4b** (3.40 g, 14.5 mmol) to a 100 mL three necked round bottomed flask under an argon atmosphere at 0°C. The reaction vessel was evacuated three times and dry DMF (25mL) was added to the vessel under pressure. CDI (4.71g, 29.0

mmol) was added rapidly via a powder addition funnel with vigorous stirring and was accompanied by copious frothing and the formation of the partially soluble diimidazolide. The slurry was allowed to warm to room temperature and HPMA (1) (4.16 g 29.0 mmol), dissolved in 10 mL of DMF, was added to the reaction from a syringe. The reaction was allowed to stir for 15 hours during which time the precipitate dissolved. TLC of the reaction mixture indicated complete conversion of the HPMA (10:90 methanol/CHCl3 Rf **5b** = 0.55). The reaction was diluted with CH2Cl2 (300 mL) and was washed with 1M NaH2PO4, (2-75 mL), NaHCO3 (2-75 mL) and of brine (100 mL). The organic layer was dried over Na2SO4. The solvent was removed *in vacuo* (T < 35°C) to yield a light yellow oil. The material was purified by flash chromatography on a Si-gel column (6 cm i.d. by 20 cm) eluting with CH2Cl2 followed by 2-propanol/CH2Cl2. Fractions containing pure product were combined and the solvent removed *in vacuo* (T < 35°C) to yield a colorless oil. Yield of **5b**: 5.83 g (83%). 1H NMR (CDCl3): 1.23 (d, J = 6.4 Hz, 6H), 1.92 (s, 6H), 2.74 (s, 4H), 3.21-3.28 (m, 2H), 3.55-3.62 (m, 2H), 4.54 (dd, 4H $J1$ = 10 Hz $J2$ = 3.2 Hz), 5.01-5.12 (m, 2H), 5.31 (d, J=1.0 Hz, 2H), 5.67 (d, 2H, J = 1 H2), 6.15-6.25 (m,2H); 13C NMR ($CDC1_3$): 17.64, 18.70, 28.51, 28.54, 43.95, 43.01, 61.45, 71.90, 119.92, 119.94, 139.86, 167.42, 167.46, 168.66, 172.026, 172.07. HRMS (FAB+) Calcd for (M+H) C22H33N2O10 485.2057 found, 485.2123.

Example 3. Synthesis of symmetrical biodegradable cross-linker HPMALacLacSuc

Preparation of (1S)-1-[benzyloxycarbonyl]ethyl (2S)-2-hydroxy propionate (BnLacLacOH). BnLacLacOH was prepared by the acid catalyzed ring opening of L-lactide. A 250 mL round bottomed flask was charged with L-lactide (15.0 g, 104 mmol), benzyl alcohol (12.4 g, 114 mmol) and camphor sulfonic acid (139 mg, 624 µmol) along with dry benzene (100 mL). The reaction was refluxed under argon for 36 hours. TLC

indicated that the reaction had consumed most of the *l*-lactide (THF/hexanes/EtOH 45:45:10 Rf lactide = 0.1 (phosphomolybdic acid stain)). The reaction was washed with 200 mM NaHCO3 (2-50 mL), dried over Na2SO4 and the solvent was removed *in vacuo*. The resultant clear oil was fractionally distilled under high vacuum (30 mtorr) using a vacuum-jacketed Vigreux column. The product was collected in a fraction between 108 and 115°C. Yield of BnLacLac0H: 19.8 g (69%) 1H NMR (CDCl3) 1.40 (d, 3H *J*=6.8 Hz), 1.49 (d, 3H *J*=3.8 Hz), 3.00 (br, 1H), 4.28-4.38 (q, 2H *J*=6.8 Hz), 5.10-5.23 (m, 2H), 7.30-7.4 (m 5H) 13C NMR (CDCl3): 16.79, 20.33, 66.68, 66.82, 67.19, 69.26, 128.20, 128.48, 128.59, 135.05, 170.09, 175.00. HRMS (FAB+) Calcd C13H16O5 (M+H) 253.0998 found 253.1066.

Preparation of (1S)-1-({(1S)-1[benzyloxycarbonyl]ethyl}oxy carbonyl)ethyl(1S)-1-({(1S)-1-[benzyloxycarbonyl]ethyl}oxy carbonyl)ethyl butane-1,4-dioate (BnLacLacSuc). BnLacLacOH (4.00 g, 15.9 mmol), pyridine (1.32 mL, 16.4 mmol) was dissolved in dichloromethane (50 mL) and cooled to 0°C under a N2 atmosphere. To this mixture was added succinyl chloride (0.90 mL, 8.2 mmol) over a period of 20 minutes. The reaction vessel was allowed to warm to 25°C, and was stirred for 3 hours. TLC indicated the reaction had nearly reached completion and an additional aliquot of succinyl chloride was added (0.5 mL, 4.5 mmol). The reaction was stirred for 1 hour more. The reaction was diluted with CH2Cl2, (50 mL), poured into water, and washed with 2N HCl (2-50 mL), water (2-50 mL), 2 M NaHCO3, (100 mL) and brine (50 mL). The CH2Cl2 phase was then dried over Na2SO4, and concentrated *in vacuo* to a bronze-colored oil. Yield of BnLacLacSuc: 35.8 g (85%). An analytically pure sample of 9 was prepared by flash chromatography on silica gel (230-400 mesh) using 30:70 ethyl acetate hexane. [∝]D= -71.60, (c=1.0, CHCl3); 1H NMR (CDCl3): 1.48 (d, 6H, *J* = 7.0 Hz), 1.50 (d, 6H, *J* = 7.1

Hz), 2.60-2.70 (m, 4H), 5.06-5.24 (m, 8H), 7.28-7.36 (m, 1 H). 13C NMR (CDCl3,): 16.38, 16.50, 28.36, 66.86, 68.36, 68.87, 127.98, 128.24, 128.37, 134.92, 169.74, 169.83, 171.29.

Preparation of (1S)-1-({(1S)-1[benzyloxycarbonyl]ethyl}oxy carbonyl) ethyl(1S)-1-({(1S)-1-[benzyloxycarbonyl]ethyl}oxycarbonyl) ethyl butane-1,4-dioate (BnLacLacSuc). LacSuc (4a) (19.1 g, 73.0 mmol) was dissolved in CH2Cl2 (75 mL) and cooled to 0°C under N2 atmosphere. CDI (26.0 g, 161 mmol) was then added to the reaction vessel. Much bubbling of CO2 gas was observed. The reaction vessel was allowed to warm to 25°C, and then benzyl (S)-(-)-lactate (25.7g, 143 mmol) was added. The reaction was stirred at 25°C for 1 hour, and then washed with 2N HCl (2-100 mL), water (100 mL), 10% NaHCO3 (2-100 mL), and brine (100 mL). The CH2Cl2 phase was then dried over MgSO4, and concentrated *in vacuo* to a bronze-colored oil. Yield of BnLacLacSuc: 35.8 g (85%). An analytically pure sample of BnLacLacSuc was prepared by flash chromatography on silica gel (230-400 mesh) using 30:70 ethyl acetate/hexane. [∝]D= -71.4, (c=1.0 CHCl3) 1H NMR (250 MHz, CDCl3): 1.48 (d, 6H, J = 7.0 Hz), 1.50 (d, 6H, J = 7.1 Hz), 2.60-2.70 (m, 4H), 5.06-5.24 (m, 8H), 7.28-7.36 (m, 1 H). 13C NMR (62.9 MHz, CDCl3): 16.38, 16.50, 28.36, 66.86, 68.36, 68.87, 127.98, 128.24, 128.37, 134.92, 169.74, 169.83, 171.29. Anal. Calcd. for C30H36O12: C, 61.43; H, 5.84. found: C, 61.47; H, 6.01.

Preparation of HOLacLacSuc: HOLacLacSuc was prepared by hydrogenation of BnLacLacSuc (35.8 g, 60.8 mmol) over 12.9 g Pd-C (10% wt. Pd, Degussa type; 6.08 mmol Pd) in 100 mL 2-propanol/ethyl acetate (2:1). Positive hydrogen pressure was maintained using a gas dispersion tube for 4 hours at 25°C and then under a balloon of hydrogen for 2 days. The Pd-C was then removed by filtration, and the solvent was removed *in vacuo*. The crude product was purified by crystallization of its dicyclohexylamine salt as follows:

Dicyclohexylamine (24.2 mL, 122 mmol) was added to the crude diacid dissolved in 200 mL 50% ethyl acetate/hexane at 25°C. Crystallization was induced by cooling to -78°C for 16 hours. The white solid was washed with 30 mL portions of 50% ethyl acetate/hexane. Concentration of the mother liquor allowed isolation of a second crop. The first and second crop were combined to give a total yield of 21.4 g ([∝]D= -42.5, c = 1.0, CHCl3). The dicyclohexylamine salt was dissolved in 25 mL water/ethanol (4:1) and subjected to strong cation exchange chromatography (Dowex 5OX4-400) to regenerate the dicarboxylic acid form. The fractions containing the pure diacid were saturated with NaCl and extracted with 3- 100 mL portions of ethyl acetate. The combined organic phases were dried over MgSO4, and concentrated *in vacuo*. The light yellow, viscous oil was then heated to 65°C under vacuum (0.5 mm Hg) to remove residual solvent. Yield of **6a** LacLacSuc: 9.31 g (38%). ([∝]D= -86.2 , c = 1.0, CHC13) 1H NMR (250 MHz, CDCl3): 1.55 (d, 6H, *J* = 7.1 Hz); 1.56 (d, 6H, *J* = 7.1 Hz); 2.70-2.80 (m, 4H); 5.09-5.22 (m, 4H); 11.07 (b, 2H). 13C NMR (62.9 MHz, CDCl3): 16.44, 28.42, 68.50, 170.00, 171.61, 175.75.

Preparation of HPMALacLacSuc (7a). HOLacLacSuc, **6a** (2.01 g, 4.92 mmol) was dissolved in 10 mL dichloromethane and cooled to 0°C under N2 atmosphere. The reaction vessel was then charged with carbonyldiimidazole (1.78 g, 11.0 mmol). Much bubbling of C02 gas was observed. The reaction vessel was allowed to warm to 25°C, and then HPMA (1.43 g, 10.0 mmol) was added. The reaction was stirred at 25°C for 2 hours, and then washed with 3-10 mL portions of 5% citric acid solution, 10 mL water, 10 mL 10% NaHCO3, and 10 mL brine. The dichloromethane phase was then dried over MgSO4, and concentrated *in vacuo* to a light yellow, viscous oil. Yield of **7a**: 2.55 g (79%). Although the purity was >90% by TLC and NMR, the purity could be improved by flash chromatography. Elution on 400 mL silica gel (230-400 mesh) using 3%

methanol/dichloromethane resulted in 2.20g ((68%) of 7a HPMALacLacSuc). ([∝]D= -24.9, c = 1.0, CHCl3) 1H NMR (250 MHz, CDCl3,): 1.23 (d, 6H, *J* = 6.4 Hz); 1.39-1.54 (m, 12H); 1.92-1.93 (m, 6H); 2.63-2.78 (m, 4H); 3.18-3.37 (m, 2H); 3.55-3.67 (m, 2H); 4.91-5.11 (m, 6H); 5.29-5.32 (m, 2H); 5.68 (d, 2H, J = 9.5 Hz); 6.28-6.33 (m, 2H); 13C NMR (62.9 MHz, CDCl3; several peaks exhibited duality which is due to diastereomers): 16.23, 16.44, 16.50, 17.11, 17.19, 18.31, 28.30, 43.41, 43.63, 68.34, 68-51, 69.58, 71.24, 71.36, 119.47, 119.60, 139.40, 139.49, 168.20, 168.38, 169.46, 169.89, 170.16, 170.45, 171.26.; HRMS(FAB+) Calcd MH+ C30H44N2O14 657.2839, found 657.2849.

Example 4. Synthesis of symmetrical biodegradable cross-linker HPMAGlyGlySuc(7a)

Preparation of di({[benzyloxycarbonyl]methyl}oxycarbonyl)methyl butane-1,4-dioate (BnGlyGlySuc). HOGlySuc (4b) (3.50g 14.95 mmol) was dissolved in CH2Cl2 (30 mL) and anhydrous DMF (60 mL) and cooled to 0°C under argon atmosphere in a three necked flask equipped with a stir bar and a powder addition funnel. The reaction vessel was then charged with CDI (4.85 g, 30.0 mmol) via a powder addition funnel. Upon the addition of the CDI the reaction frothed copiously. The insoluble diimidazolide formed a thick precipitate. The reaction vessel was allowed to warm to 25°C, and then benzyl glycolate (3.82 mL, 30.0 mmol) was added via a syringe in anhydrous DMF (10 mL). The reaction was allowed to run overnight at 25°C. As the reaction proceeded, the reaction mixture slowly became less viscous. The reaction was diluted with CH2Cl2 (500 mL) and was washed with 1M NaH2PO4 (2-100 mL), NaHCO3 (2-100 mL), and brine (100 mL). The organic layer was dried over Na2SO4, and the solvent was removed *in vacuo*. Yield of 4b: 7.67 g, 98% (a light yellow crystalline solid). The compound was purified by flash

chromatography on a 4.5 cm i.d. by 12 cm column over silica gel. The sample was loaded in 2:1 CH2Cl2 /hexanes (100 mL) eluted with of the same (200 mL), of CH2Cl2 (200 mL), of 1:99 THF/CH2Cl2 (200 mL), and finally with THF/CH2Cl2 (3:97). The fractions containing pure product were combined and the solvent removed *in vacuo* to yield 26 as a pure crystalline solid. Yield of 4b: 6.57 g (83%). 1H NMR (MHz, CDCl3): 2.78 (s, 4H), 4.71 (s, 4H), 4.73 (s, 4H), 5.18 (s, 4H), 7.33-7.39 (m, 10H); 13C NMR CDCl3) 28.65, 60.66, 61.21, 67.42, 128.55, 128.74, 128.78, 135.00, 167.03, 167.22, 171.40; Anal. Calcd. for C26H22O14 C, 55.92; H, 3.97; Found: C, 55.64; H, 4.01 .

Preparation of 2-{2-[3-({[Carboxymethyl)oxycarbonyl]methyl} oxy carbonyl)propanoyloxy]acetyloxy}acetic acid, HOGlyGlySuc (6b). Compound **6b** was prepared by suspending **4b** (4.58 g, 8.61 mmol) in 1:1 2-propanol/CH2Cl2 (250 mL) in the presence of Pd/C (2.0 g, Degussa type). The reaction mixture was placed on a Parr hydrogenator at 50 PSI for 18 hours at which time uptake of hydrogen gas had stopped. The reaction was filtered through celite to remove the catalyst, and the solution was concentrated *in vacuo*, resulting in a white solid. The solid was triturated with diethyl ether and dried further yielding a white solid. Attempts at purification by recrystallization of the dicyclohexylamine salt resulted in complex mixtures upon trying to remove the amine by semi-aqueous ion exchange. This *was* likely due to the instability of this compound. However the 1H NMR spectrum of the triturated product was adequate with a purity > 95%. Yield of **6b**: 2.87 g (93%): 1H NMR (d6-DMSO): 2.80 (s, 4H), 4.66 (s, 4H), 4.77 (s, 4H), 13C NMR (d6-DMSO): 29.31, 61.34, 61.91, 167.92, 169.57, 171.97. HRMS (FAB) Calcd for (M+H) C12H13O12, 349.0485 found 349.0403.

Preparation of di[({[1-methyl-2-(2-methylprop-2-enoylamino) ethyl] oxycarbonyl}methyl)oxycarbonyl]methylbutane-1,4-dioate (HPMAGlyGlySuc) (7b). The cross-linker HPMAGlyGlySuc **7b** was prepared by adding **6b** (1.50 g, 4.28 mmol) to 100 mL three-necked round bottomed flask under an argon atmosphere at 0°C. The reaction vessel was evacuated three times and dry 1:1 DMF/CH2Cl2 (35mL) was added to the vessel under positive argon pressure. The CDI (1.39 g, 8.57 mmol) was added rapidly with vigorous stirring via a powder addition funnel and was accompanied by frothing and the formation of the insoluble diimidazolide. The slurry was allowed to warm to room temperature, and HPMA (1.23g, 8.57 mmol) dissolved in DMF (10 mL) was added to the reaction through a syringe. The reaction was allowed to stir for 10 hours during which time the precipitate

dissolved. TLC of the reaction mixture indicated complete conversion of the HPMA (10:90 methanol/CHCl3 Rf 7b = 0.73). The reaction was diluted with CH2Cl2 (200 mL) and was washed with 1M NaH2PO4 (2-50 mL), NaHCO3 (2-50 mL) and brine (100 mL). The organic layer was dried over Na2SO4. The solvent was removed *in vacuo* (T < 35°C) to yield a clear oil. The material was purified by flash chromatography on a 6 cm i.d. by 20 cm silica gel column eluting with CH2Cl2 followed by 3:97 2-propanol/CH2Cl2. Pure fractions were combined and the solvent removed *in vacuo* (T < 35°C) to yield a colorless oil. Yield of 7b: 2.08g (81%): 1H NMR (CDCl3): 1.23 (d, 6H J = 6.3 Hz), 1.89 (s, 6H), 2.73 (s, 4H), 3.21-3.30 (m, 2H), 3.53-3.62 (m, 2H), 4.52-4.8 (m, 8H), 5.01-5.17 (m, 2H), 5.28 (s, 4H), 5.64 (s, 4H), 6.23-6.35 (b, 2H), 13C NMR °CDCl3): 17.45, 18.62, 43.80, 60.64, 61.54, 72.12, 119.74, 139.84, 166.75, 167.49, 168.67, 171.50. HRMS (FAB+) Calcd for C26H36N2O14 (M+H) 601.2167 found 601.2219.

Example 5. Synthesis of degradable hydrogels with TMED initiation.

Biodegradable hydrogels are synthesized by free radical polymerization of the biodegradable cross-linkers and other monomers described herein using the APS/ TMED couple.

The vinyl groups on the terminus of the cross-linking structure can be used to form a gel network structure. Gels were synthesized using the ammonium persulphate (APS) N,N,N',N'-tetramethylethylenediamine (TMED) couple as the free-radical initiator system. This system proved very useful in the synthesis of clear isotropic gels, without having to degas the polymerization reactions. The gels in this section were made at a mole feed ratio of 1.5 mole % cross-linker, as a copolymer with 98.5 mole % HPMA. Before the gels were polymerized, three 1.0 mL plastic syringes to be used as a slab gel template were silylanized by briefly incubating them in a heptane solution containing Sigmacote and oven drying at

90°C. Also, three 8 cm lengths of 25 gauge tungsten wire were silylanized for use in the gel making process and each was threaded through 7mm Suba Seal rubber septa.

The procedure to form gels was as follows: A 7 mL test tube was charged with HPMA (2.115 g, 14.8 mmol [HPMA] final ~ 5 M), the oily compound **5b** (HPMAGlySuc) was adsorbed to the end of a tared spatula (109.0 mg, 0.225 mmol, [XL]final =0.075 M). The end of the spatula was placed in the test tube and 1.5 mL of DI water was added to the mixture. The cross-linker was dissolved in the mixture by rapid rotation of the spatula and gentle bath sonication.

The dissolution of the HPMA has a negative heat of solution, but the mixture should not be warmed above room temperature. To this solution was added a solution of APS in water (99 mg, 0.438 mmol, 166 μL of a 2.63 M solution, [APS] final=0.143 M). This was again agitated until homogeneous. To this mixture was added TMED to initiate the polymerization (49mg, 0.429 mmol, 204 μL of a 2.10 M solution of TMED adjusted to pH 7 with HCl). In this preferred embodiment the concentration of TMED must be approximately 0.15 M or greater. It was important to control the pH of the TMED because TMED solutions in water are basic enough to cause significant degradation of the hydrolytically reactive cross-linker. Immediately after the TMED was added the mixture of monomers and APS was vigorously mixed on a vortexer for 15 seconds and then drawn into the 1.0 mL plastic syringes by plunger aspiration. The syringe acts as a mold for gel formation. The syringes were inverted, and the tungsten wires were inserted into the gel through the opening so that it runs through the center of the forming 1.0 mL gel cylinder.

The wire was held in place by a septa which was placed over the tip of the syringe, as the solution polymerized. This formed a hole in the center of the cylinder, which was

later used as a place to insert a wire hanger for the initially brittle and finally fragile gel, in order to measure its swelling and degradation kinetics as a change of mass with time. Gelation occurred within one to five minutes and the syringe was allowed to sit for 4 hours at room temperature. At this point the wire was removed from the center of the solid, and the end of the plastic syringe was removed with a razor blade. The plunger was then used to extrude the gel from the syringe in 100 μL increments which were cut into small cylinders as they hung out from the end of the syringe. The gels were then placed on tared wire holders and the initial mass of the assembly was determined. The resulting clear isotropic gels had the composition of poly(HPMA-co-HPMAGlySuc) 98.5:1.5. The gels were then incubated in pH 5, 100 mM sodium acetate buffer for 24 hours. (Ester hydrolysis is very slow at this pH). They were then charged into vials of differing pH to study the degradation kinetics.

The gels of the four different compositions contained the following amounts of cross-linkers:

Compound	MW	Mole %	XL moles	Mass (mg)
HPMASuc	366.38	0.015	2.25E-04	82.4
HPMALacSuc	512.5	0.015	2.25E-04	115.3
HPMAGlySuc	484.51	0.015	2.25E-04	109.0
HPMAGlyGlySuc	600.58	0.015	2.25E-04	135.1

Example 6. Synthesis of biodegradable hydrogel using AIBN thermal initiation.

To a 10 mL round bottomed flask was charged HPMALacLacSuc 7a (60.75 mg, 125 mol) and azobisisobutryonitrile (free radical initiator) (4.0 mg). To this was added 1.0 mL of a 1:1 methanol:water mixture. The contents were dissolved and degassed under N2 for

0.5 hours followed by 5 minutes in a bath sonicator while bubbling in a stream of N2. The mixture was charged in 300 µl aliquots into 36 X 5.0 mm glass tubes which have been evacuated and capped with rubber septa. The tubes were placed in a 60°C bath overnight. The next morning the polymer gel had formed. It was removed from the glass tube under vacuum and cut into 2 X 5 mm disks. The disks were incubated in water for two days. The water was changed at 8 hour intervals to remove any water soluble monomer or reaction byproducts.

Example 7. Degradation of Biodegradable Hydrogels.

Measuring the degradation of the gel network

The gels were placed in 15 mL vials containing 10 mL of one of four buffers at pH=0.73, 4.0. 7.3 and 9.0. The masses of wire holders were determined before the gels were placed on them. The original mass of the gel in its relaxed state was also known by subtraction of wire's weight from the total mass of the assembly. The original dry mass of the gel was determined by drying three gels in their relaxed state from each composition and determining the dry mass of the gel. These values were then used to calculate the inverse of the volume fraction of polymer in the gel (Qv) respectively, using the densities of the polymer and water. The incubation solutions were changed each time the gel was weighed. The gels were incubated in a gyratory water-bath shaker (New Brunswick Scientific, New Brunswick, NJ). The temperature was regulated to be 37 ± 2°C and the shaker was set to 30 rotations per minute.

Explanation of the order of the rates of degradation for different cross-linkers

Hydrogels are cross-linked structures composed of elastic networks of water-soluble polymers. The maximum degree of swelling is limited by the network elasticity. So as the gel's network structure degrades the cross-link density decreases and the network becomes

more elastic. This allows the network to swell further as it imbibes more water. This swelling results in an increase in the volume fraction of water and a corresponding decrease in the volume fraction of polymer. The property of the change of volume of the polymer network can be measured by weighing the gel at different time points.

Since the swelling is related to network cross-link density by weighing the macroscopic gels at various times throughout their swelling one can obtain information about the change in cross-link density and thus the rate of degradation. The cross-linkers in the gel degrade hydrolytically by the action of the two hydrolytically active components of water: the hydronium and hydroxide ions. Therefore, the rate of degradation is strongly dependent on pH.

HPMALacSuc **5a** is electronically similar to HPMAGlySuc **5b** yet the lactic acid ester shows slower degradation than the glycolic one. This is because the lactic acid ester has a methyl group alpha to the carbonyl where the first step of ester hydrolysis takes place, which makes the lactate carbonyl more sterically hindered in **5a** than in **5b**. HPMAGlyGlySuc **7b** shows the fastest hydrolysis and swelling kinetics with complete degradation after about 5 days (See FIGS. 4 through 6).

Moreover, since we are not measuring the rate of hydrolysis of individual bonds but measuring the swelling which comes about as a result of cleaving the connection between two polymer chains, the concentration of cleavable sites comes into play. Therefore, when comparing gels cross-linked with **5b** and **7b** at the same cross-link density, compound **5b** has four potential sites of cleavage whereas **7b** has six. This increased concentration of cleavable sites may result in a difference in swelling rate depending on the relative microscopic rate constants for hydrolysis of the different bonds making up the cross-linker. In other words, cross-linkers numbers of cleavable sites should clear or degrade at a faster

rate than cross-linkers with fewer cleavage sites, with everything else equal.

Synthesis of the control cross-linker HPMASuc

Preparation of the control non-degradable (pH 7.3) cross-linker bis-1-methyl-2-(2-methylprop-2-enoylamino) ethyl -1,4-butanedioate (HPMASuc) (2). To a solution of HPMA (4.00 g, 27.9 mmol), DMAP (340 mg, 2.8 mmol) and Na2CO3 (3.26 g, 30.7 mmol) in CH2Cl2 (100 mL) at 0°C was added succinyl chloride (1.54 mL, 13.97 mmol) dropwise. The reaction was allowed to warm to 25°C and stirred for 8 hours at which time another aliquot of succinyl chloride was added (0.61g, 4 mmol). The reaction was allowed to stir for another 4 hours. The reaction mixture was poured into 50 mL of water and filtered through activated carbon. The mixture was then washed with 1 M NaH2PO4 (50 mL), sat. NaHCO3 (50 mL) and brine (100 mL). The organic phase was then dried over Na2SO4 and concentrated *in vacuo* to a tan residue. This was purified by flash chromatography in 15:85 2-propanol/CHCl3 on a 3 i.d. by 20 cm column of Si-gel. Yield of 2 3.51g (68%): mp 103-105°C, 1H NMR CDCl3): 1.24 (d, 6H, J = 6.3 Hz); 1.93 (d, 6H, J = 0.6 Hz), 2.54-2.65 (m, 4H), 3.31-3.38 (m, 2H), 3.52-3.60 (m, 2H), 4.98-5.05 (m, 2H), 5.31 (d, 2H, J = 0.4 Hz), 5.66 (s, 2H), 6.23 (b, 2H). 13C NMR °CDCl3); (several peaks exhibited duality which is most likely due to diastereomers) 17.48, 18.51, 29.38, 43.81, 43.87, 70.48, 70.62, 119.50, 119.56, 139.78, 168.48, 172.24. Anal. Calcd. for C18H28N2O6: C, 58.68; H, 7.65; N, 7.60. found: C, 58.71; H, 7.72; N, 7.48.

Example 8. Release of a soluble macromolecule from a degrading network and degradation of a polymer network labeled with a chromophoric agent.

Gels were formed by the same method as above, but in this case other compounds were included during the preparation of the gels to study the release of small molecules from the network. In one case, the network itself was labeled with a polymerizable derivative of

tetramethyl rhodamine (TMRAHMAm) in order to show the release (degradation) of the network itself (see FIG. 6) (TMRAHMAm is a 5 or 6-carboxytetramethlrhodamine dye linked to a methacryloyl group via a 1,6-diaminohexane linker). In the other case fluorescent rhodamine labeled albumin (Molecular Probes, Eugene OR) was included in the uncharged network to show diffusive release of a macromolecule from the network (see FIG. 7).

To a 3 mL test tube was charged TMRAHMAm (3.0 mg, 5.0 μmol; 30 μL of a 100 mg/mL solution in CHCl3) which was then placed under a 7 mtorr vacuum for 3 hours. To another 3 mL test tube, 4.5 mg of 5+(6)-carboxytetramethylrhodamine labeled albumin (Molecular probes) was added. To a third 7.0 mL test tube HPMA was added (1.692 g, 11.8 mmol, [HPMA] final ~ 5 M), the oily compound 7b (HPMAGlyGlySuc) was adsorbed to the end of a tarred spatula (108.0 mg, 180 μmol, [XL]final = 0.075 M). The end of the spatula was placed in the test tube and 1.2 mL of DI water was added to the mixture.

The cross-linker was dissolved in the mixture by rapid rotation of the spatula and gentle bath sonication. HPMA has a negative heat of solution but the mixture should not be warmed above room temperature. To this solution was added a solution of APS in water (80 mg, 0.350 mmol, 79 μL of a 2.63 M, [APS]final=0.143 M). This was again agitated until homogeneous. This viscous mixture was separated into 3-890 μL aliquots. One was mixed with the polymerizable dye (TMRAHMAm) of the first test tube from above, and the other with the fluorescent albumin. All resulting monomer mixtures were thoroughly homogenized. To each of these three 890 μL mixtures was added an aliquot of TMED to initiate the polymerization (13.2 mg, 114 μmol, 54 μL of a 2.10 M solution of TMED adjusted to pH 7 with HCl, 244 mg TMED freebase/mL).

All mixtures were mixed for 15 seconds and then placed in the syringe template as described in Example 5, with each solution having a final solid volume of about 850 μL.

The gels were then allowed to polymerize for 4 hours after which time they were extruded and cut into slices. The gels were weighed, attached to wires and were placed in separate vials for the release studies. The dye labeled gels were incubated in water for two days to allow any unreacted monomer to diffuse out of the network. The gels were placed in 15 mL of buffer solutions at pH 4, 7, and 9. All solutions were incubated at 37°C on a temperature-regulated orbital-shaking bath at 30 rpm.

Gels were suspended in buffers of different pH's. All buffers were adjusted to the same ionic strength. The release of the rhodamine labeled albumin and the rhodamine labeled HPMA was monitored at 550nm. 750 μL of the sample was removed from the vial and periodically measured on a spectrophotometer. Release values were normalized to the maximum amount released. Results of these studies are shown in Fig. 6 and Fig. 7.

Explanation of the release data

The examination of the release of macromolecules entrained in the polymer network provides another way to study the performance of these materials. In this section, the degradation of the network is ascertained by analysis spectrophotometrically through the release of the HPMA polymer backbone itself by labeling it with the polymerizable dye TMRAHMAm. Moreover, the release of a model macromolecular solute (TMRA-albumin, molecular weight of ~66,000 Da.) from the network is measured spectrophotometrically.

FIG. 6 displays a photograph of three different gels in pH 7.3 buffer made with HPMASuc (15 days), HPMAGlyGlySuc (4 days) and HPMAGlyGlySuc (8 days) respectively, which were co-polymerized with the chromophoric label and HPMA. FIG. 6 displays not only the different degrees of swelling but also the release of rhodamine labeled HPMA into the solution at a given time versus control.

FIG. 7 shows the release curve for rhodamine labeled HPMA polymer backbone as well as the corresponding swelling data. The release of rhodamine labeled HPMA largely occurs to the greatest extent at the onset of complete degradation of the polymer. In

contrast to the release of the polymer backbone, the release of the globular macromolecule BS albumin more closely follows the swelling of the network. As shown, no initial large burst release of labeled BS albumin was observed.

Example 9. Synthesis of anionic slab gels and loading of DX (doxorubicin).

Synthesis of gels containing methacrylic acid.

The gels in this section were made at a mole feed ratio of 1.45 mole percent cross-linker as a copolymer with HPMA (95.4 mole %) and methacrylic acid sodium salt (3.18 mol%). Before the gels were polymerized, three 1.0 mL plastic syringes to be used as a slab gel template were silylanized with Sigmacote by briefly incubating them in the heptane solution and oven drying at 90°C (see Example 5). Also, three 8 cm lengths of 25 gauge tungsten wire. The procedure to form gels is as follows: to a 7 mL test tube is charged HPMA (564.1 mg, 0.00394 mol, [HPMA] final ~5 M), the cross-linker (6.00E-05 mol, [XL] final = ~0.075M) and the sodium salt of methacrylic acid (42 mg 3.89E-04 mol) are charged into the same vial with the HPMA and 0.4 mL of DI water is added to the mixture. The components of the mixture are dissolved by agitation and gentle bath sonication at 15°C. The dissolution of the HPMA is retarded by its negative heat of solution but the mixture should not be warmed above room temperature. To this solution was added 44.4 μL of a 2.63 M solution of (APS) in water (27 mg, 1.17E-04 mol, [APS] final=0.143M). This is again agitated until homogeneous. To this mixture is added 55 μL of a 2.10 M solution of TMED to initiate the polymerization (13.3 mg, 1.14E-04 mol, TMED solution adjusted to pH 7). It is important to control the pH of the TMED because TMED solutions in water are basic enough to cause significant degradation of the hydrolytically reactive cross-linkers. Immediately after the TMED is added the mixture of monomers and APS is vigorously mixed on a vortexer for 15 seconds and then drawn into the 1.0 mL plastic syringes by plunger aspiration which acts as a mold for the forming gel (see above for a

description of gel processing).

For gels of the three different compositions synthesized the following amounts of cross-linkers were used in addition to the materials described above.

Compound	MW	Mole fraction	Moles	Mass (mg)
HPMASuc	366.38	0.0136	0.00006	22
HPMAGlySuc	484.51	0.0136	0.00006	29
HPMAGlyGlySuc	600.58	0.0136	0.00006	36

Loading with Doxorubicin

4 hours after initiation of polymerization, the gels were cut into approximately equal volumes (100μL, ~ 100 mg) and the unloaded masses were determined for the gels in the rubbery state. Each gel was placed in 1.9 mL of a 2.0 mg/ml solution of doxorubicin hydrochloride which was buffered to pH 7.4 with 5 mM TRIS buffer. The gels were agitated with the solution for 4 days at room temperature on a temperature-regulated orbital-shaking bath at 30 rpm. As the red doxorubicin was taken up into the gels the gels became red. The solution around the gels became depleted of doxorubicin due to the ion exchange of doxorubicin for the sodium counterions. At longer temperatures and times earlier examples (see Fig. 4 - Fig. 7) show that this loaded hydrogel will degrade and release the loaded drug. This rate of release can be controlled by the choice and amount of degradable cross-linker for, as shown in Fig. 4 and Fig. 5, cross-linkers with more ester groups and more glycolic acid residues will degrade faster. Also, lower cross-linker concentrations mean fewer links to degrade for a given swelling volume (Qv). (Note: under physiological conditions and over time the data in Fig. 4-Fig. 7 show that the loaded hydrogel will degrade and release the loaded drug. Additionally, the rate of release can be controlled by the choice of degradable cross-linker, for cross-linkers with more ester groups and glycolic acid groups

degrade faster.)

Example 10. Method for making a biodegradable water absorbant device.

As discussed earlier the preferred embodiment of a network polymer for use as a degradable water absorbent will include ionomeric monomers which bring ions and water into the gel network. Below is a description of the method to make a highly charged gel of these cross-linkers.

To a 5 mL test tube was charged acrylic acid (675 µL, 9.85 mmol), water (1120 µL) and HPMAGlySuc (73 mg, 0.15 mmol) (**5b**). The cross-linker was weighed into the mixture as described earlier. The mixture was homogenized and APS was added (66.6µL, 0.175mmol) from a 2.43 M solution in water. This solution was again mixed. To this solution was added TMED (137 µL, 0.287 mmol) from a 2.10 M pH 7.0 solution in water. The mixture was vortexed rapidly for 15 seconds and the polymerizing solution was charged into two 1.0 mL syringes that acted as a mold for the polymerization. The syringes were allowed to sit for four hours. The gel was removed from the syringe and cut into pieces (~100 µL cylinders). The mass of the cylinder was recorded and placed in a 20 mL vial containing 18 mL of PBS (phosphate buffered saline) at pH 7.4. The gels were incubated overnight with buffer. The next day the buffer was changed twice in order to keep a constant external pH as the gel was charged. After incubating in PBS for 3 days the gel has swollen with water to approximately 20 times the total initial polymer volume.

The salt form of the gel will be synthesized and the gel material processed into smaller pieces either before or after drying. The dry gel pieces would then be incorporated as one component in an absorbable layer of the absorbent device. Generally the pieces should to small so as to increase the surface area of the gel and therefore to increase the rate at which water would be absorbed by the gel material.

Example 11. General Method for synthesizing the cross-linker.

Those skilled in the art of organic synthesis will be aware of the general

considerations in designing cross-linkers of this class. Generally, if any alcohol groups are present in the poly-acids used they must be protected unless it desired that they react with the activated acids to be used in the formation of the oligo-ester. Generally, the synthesis must be performed under anhydrous condition except when performing acid or base washes of water immiscible organic solvents where the cross-linker or intermediate largely partitions into the organic phase. If the materials are to be used in an aqueous environment it is generally best to keep the acid in the anionic form only a few units above its pKa. This is due to the well-known effect of inhibition of attack of hydroxide of electrophilic site with nearby negative charges. In the most preferred cases the cross-linkers are constructed by adding a protected degradable piece to a polyacid. In a preferred embodiment the degradable piece contains a nucleophilic moiety and a protected acidic moiety, *e.g.* benzyl lactate. The protecting groups are removed under appropriate conditions known to those skilled in the art. The activation and reaction with a protected bifunctional degradable molecule can be repeated on the molecule as many times as desired. Alternatively, the final step of the synthesis can be accomplished by terminating the molecule with reactive groups that are later used to cross-link polymer filaments. The preferred embodiment of the protecting group are groups that can be removed under neutral anhydrous conditions such as the benzyl protecting group. The next preferred protecting groups are ones that can be removed with anhydrous acids or bases such as the BOC or MEM protecting groups.

Example 12. Preparation of HydLacSuc (8a).

Preparation of di[N-carbobenzoxy-N'-hydrazidooxycarbonyl]ethyl butane-1,4-dioate (BnHydLacSuc). To a 25 mL round-bottomed flask was charged 4a (262 mg, 1.00 mmol), THF (2.0 mL), and pyridine (162 μL, 2.00 mmol). The flask was placed on an ice bath and to the reaction was added isobutyl chloroformate (260 μL, 2.0 mmol). The reaction was allowed to stir and carbobenzyloxyhydrazide was added (380 mg, 2.3 mmol).

The reaction was allowed to stir overnight. The white solid was dissolved in ethyl acetate and washed with 1M HCl (2-5 mL), water (5 mL) and saturated NaHCO3 (5 mL). The organic layer was dried over MgSO4. The solvent was removed *in vacuo* resulting in a white solid. Yield of BnHydLacSuc: 392 mg (62%).

Preparation of di[N-hydrazidooxycarbonyl]ethyl butane-1,4-dioate (HydLacSuc) (8). To a 5 mL pressure tube was BnHydLacSuc (279 mg, 0.5 mmol), Pd-C (Degussa Type, 10% Pd, 50% H20) (600 mg) and cyclohexene (1.25 mL, 12.5 mmol) and MeOH/DMF (1:1, 1.25 mL). The reaction was heated to 60°C for 3 hours. Evolution of CO2 was observed. The Pd-C was removed by filtration and the solvent was removed in vacuo resulting in an oil (**8a**).

Example 13. Preparation of HEMAGlyAdp

Preparation of di[benzyloxycarbonyl]methyl hexane-1,6-dioate (BnGlyAdp). Compound BnGlyAdp was synthesized by methods similar to those described for BnGlySuc, compound **3b**, by dissolving benzyl glycolate (9.08 g. 54.6 mmoles) and pyridine (4.42 mL., 54.6 mmoles) in 150 mL CH2CH1 at 0°C and adding adipoyl chloride (5.00 g, 27.3 mmoles) via a syringe while stirring under nitrogen atmosphere. The reaction was allowed to warm to room temperature and was stirred for 5 hours. After 5 hours, TLC (5:95 methanol/CH2Cl2 Rf = 0.63) indicated almost complete conversion, and 0.1 mL of adipoyl chloride was added. The reaction was stirred 12 more hours. The medium was then cooled to 0°C in a freezer for 2 hours to facilitate precipitation of pyridinium chloride salt (PyCl). After 2 hours, the medium was filtered through a medium porous frit funnel and the filtrate was washed with 3-100 mL water washings. The organic layer was dried over Na2S04 for 2 hours. The CH2Cl2 was stripped on a roto-evaporator to concentrate the BnGlyAdp. The material was purified by recrystallization (from 1:1 ethyl acetate/hexane). Yield of BnGlyAdp: 7.72g (64.0%). ^{1}H NMR (d, DMF): δ 1.67 (s, 4H), 2.46 (s, 4H), 4.80

(s, 4H), 5.23 (s, 4H), 7.44 (m, 10H).

Preparation of 2,3-[(carboxymethyl)oxycarbonyl]hexanoyloxyacetic acid (HOGlyAdp). Compound HOGlyAdp was synthesized by methods similar to those described for HOGlySuc, compound **4b**, by dissolving BnGlyAdp (5.01 g, 11.3 mmoles) in 250 mL of 2-propanol at room temperature in the presence of 1.51 g Pd/C (Degussa type). An air stone was immersed in the medium through rubber septum at the top of the flask. The medium was sparged with hydrogen gas at 1 atm. The system was isolated from air using a closed system bubbler. The medium was sparged with hydrogen gas for 12 hours. After 12 hours, the reaction mixture was filtered through celite to remove the catalyst and the reaction product was concentrated *in vacuo* resulting in a white solid. The white product was triturated with 1:1 diethyl ether/hexane. The white product was recovered by filtration through a medium porous filter funnel and then dried under vacuum in a desiccator. Yield of HOGlyAdp: 1.74 g (60.0%).

Preparation of di{[1-methyl-2-(2-ethyl)oyloxycarbonyl}methy]hexane-1,6-dioate (HEMAGlyAdp). Compound HEMAGlyAdp was synthesized by methods similar to those described for HPMAGlySuc, compound **5b**. The cross-linker HEMAGlyAdp was prepared by adding HOGlyAdp (500 mg, 1.92 mmoles) and CDI (622 mg, 3.83 mmoles) to a 50 mL boiling flask. The flask was evacuated 3 times while iteratively purging with nitrogen. The temperature of the reaction vessel was reduced from room temperature to 0°C with an ice bath, and dry DMF (5 mL) was rapidly added to the vessel under pressure with vigorous stirring via a magnetic stir bar. Addition was accompanied with frothing and the formation of a white slurry of the intermediate diimidazolide of GlyAdp. The slurry was allowed to come to room temperature and hydroxyethyl methacrylate (HEMA, 466 μL, 3.84 mmoles) was added via a syringe. The vessel was covered with aluminum foil to shield it from light and the reaction mixture was stirred under nitrogen atmosphere for 15 hours over which

time the slurry completely dissolved. TLC of the reaction mixture showed the presence of both unreacted HEMA and HEMAGlyAdp (5:95 methanol/ CH_2Cl_2 Rf = 0.80). The reaction was diluted with 100 mL CH_2Cl_2 and washed with 1M NaH_2PO_4 (pH 4.5, 2-50 mL), 1M $NaHCO_3$ (pH 8.3, 2-50 mL) and brine (2-50 mL). The organic layer was dried over Na_2SO_4. The organic layer was recovered by filtration and solvent removed *in vacuo* (T < 30°C) yielding a yellow oil that was purified by flash chromatography on a Si-gel column (5 cm id by 30 cm) eluting with CH_2Cl_2. Fractions containing pure product were combined and the solvent was removed *in vacuo* (T < 30°C) yielding a colorless oil.

Example 14. Synthesis of asymmetrical biodegradable cross-linker HPMAGlySucHPMA (9b)

Preparation of [4-(2-benzyloxycarbonyl)methyl]butane-4-oate-1-oic acid: (BnGlySuc-4-oate-1-oic acid). A three neck round bottom flask equipped with a stir bar, condenser, and septa was charged with 100 mL of DCM and 7.07 g (70.5 mmole) of succinic anhydride. Upon cooling to *ca.* 4 °C under a N_2 atmosphere, 6.0 mL (74 mmole) of pyridine and 10 mL (70 mmole) benzylglycolate (BnGly) were successively added. The mixture was gently refluxed for 4 hours then allowed to stir overnight at ambient temperature under a N_2 atmosphere. Afterwards, TLC indicated the absence of BnGly and the presence of product (5% MeOH/DCM, Rf: 0.75 for BnGly, Rf: 0.5 for BnGlySuc-4-oate-1-oic acid). Solvents were removed *in vacuo*, and the remaining white residue was dissolved in 25 mL DCM, washed with two 50 mL portions of 1 M NaH_2PO_4, and dried with Na_2SO_4. The DCM was removed *in vacuo*, and the residue was recrystallized from 25 mL EtOAc and 5 mL hexane at –20 °C to give 12.9 g (70 %) **BnGlySuc-4-oate-1-oic acid**. mp: 86.1 – 86.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 2.69-2.75 (m, 4H), 4.67 (s, 2H), 5.19 (s, 2H), 7.34-7.35 (m, 5H), and at 8.00 (bs, 1H). ^{13}C NMR ($CDCl_3$): 28.5, 28.8, 61.0, 67.3, 128.5, 128.7, 128.75, 167.7, 171.7, and 177.8.

Preparation of (carboxymethyl)butane-4-oate-1-oic: (GlySuc-4-oate-1-oic). A 300 mL pressure vessel was charged with a magnetic stir bar, 150 mL of 2-propanol, 8.0094 g (30.08 mmole) BnGlySuc-4-oate-1-oic acid and 1.0032 g 10% Pd/C (Degussa type). After appropriate purging the vessel was pressurized to 35 psi with H_2 and allowed to stir at ambient for 48 h. The reaction mixture was filtered through celite, and TLC indicated the absence of BnGlySuc-4-oate-1-oic acid (5% MeOH/DCM). The solvent was removed *in vacuo* and triturated with EtOEt. The product was collected and dried *in vacuo* to give 5.30 g (88 %) of GlySuc-4-oate-1-oic acid: mp: 111 –113 °C; ^{1}H NMR (D_2O) : 2.70-2.74 (m, 4H), 2.77-2.81 (m, 2H), 4.72 (s, 2H). ^{13}C NMR (D_2O) 28.6, 28.7, 61.5, 172.2, 174.4 and 176.8.

Preparation of di[1-methyl-2-(2-methylprop-2-enoylamino)]ethyleneacetate-butane-1,4-dioate: (HPMAGlySucHPMA, 9b). A one necked round bottom flask equiped with a stir bar and septum was charged with 4.3210 g (24.53 mmole) Gly-Suc-4-oate-1-oic acid and 7.9565 g (49.07 mmole) 1,1'-carbonyldiimidazole (CDI). While stirring under a N_2 atmosphere, 30 mL of DCM was added dropwise over a 15 minute period with concurrent frothing and liberation of CO_2. Upon secession of CO_2 evolution, a solution of 7.073 g (49.40 mmole) of HPMA in 45 mL DCM was cannulated into the reaction vessel. The flask was protected from light and left stirring overnight under a nitrogen atmosphere. TLC indicated that absence any HPMA (5% MeOH/DCM). The solution was repeatedly washed (3-100 mL of 1 M NaH_2PO_4, 3-100 mL of 1 M bicarbonate, and 1-100 mL brine). Solvents were removed *in vacuo*, and the remaining amber oil was dissolved in 25 mL DCM. This solution was applied to small column of Si-gel (5 x 7 cm, ht. x w.) in a 150 mL, medium-frit, Buchner funnel. The product was eluted with DCM which after *in vacuo* removal gave 9.867 g (94 %) of **9b** (HPMAGlySucHPMA) as a clear, colorless oil: ^{1}H NMR ($CDCl_3$) 1.25-1.293 (2d, 6H, J= 6.4), 1.95 (s, 6H), 2.63-2.97 (m, 4H), 3.33-3.41 (m,

2H), 3.53-3.65 (m, 2H), 4.53-4.66 (m, 2H), 5.04-5.14 (m, 2H), 5.31 (s, 2H), 5.69 (s, 2H), 6.34-6.35 (b, 2H); ^{13}C NMR ($CDCl_3$) 17.64, 17.74, 17.77, 18.75, 28.89, 28.92, 29.317, 29.35, 44.01, 44.20, 61.40, 61.63, 70.84, 71.92, 119.77, 119.79, 119.90, 139.95, 140.02, 167.57, 168.71, 171.74, 172.04, 172.42, and 172.48.

Example 15. Synthesis of di[(succinimidyloxycarbonyl)methyl butane-1,4-dioate: (NHSGlySuc, 10b). A 50 mL round bottom flask equiped with a stir bar and septum, was charged with 501 mg (2.14 mmole) of **4b** (GlySuc) and 1.09 g (4.27 mmole) disuccinimidyl carbonate (DSC). The flask was topped with a rubber septum and cooled to 4 °C. While stirring under a nitrogen atmosphere, a solution of 691 *u*L (8.54 mmole) of pyridine in 10 mL of MeCN was added dropwise over *ca.* 10 minutes from a syringe with concurrent frothing and liberation of CO_2 gas. From the solution which initially formed, a white preciptitate developed after *ca* 4 hours. This mixture was stirred overnight then then centrifuged at 375 RCF for 5 minutes. The supernatant was discarded and the solid was repeatedly triturated with 2-10 mL ACN, 2-10 mL 1 M NaH_2PO_4, 2-10 mL MilliQ water and 10 mL ACN. After each trituration the mixture was centrifuged, and the supernatant was discarded. The solid were dried *in vacuo* to yield 300 mg (30 %) of **10b** (succinimidylGlySuc): mp: dec. 188 °C; ^1H NMR (DMSO-d_6) 2.66-2.73 (m, 4H), 2.82 (s, 4H), 2.60-2.80 (m, 4H), 5.15 (s, 4H). ^{13}C NMR (DMSO-d_6) 25.45, 28.01, 58.70, 169.79, 170.98, and 172.77.

Example 16. Synthesis of fluorescent-labeled, biodegradable nano-particles by emulsion polymerization. A 250 mL round bottom flask was charged with a magnetic stir bar, 110 mg (0.381 mmol) sodium dodecyl sulfate, 100 mL deionized water, 331 mg (2.312 mmol) HPMA 1, 276 *u*L (2.719 mmol) methacrylic acid, 929 uL (8.605 mmol) methyl methacrylate, 175 mg (341 mmol) HPMALacSuc **5a**, 660 mg (0.6 mmol) poly(ethylene glycol) (n = 200) monomethyl ether monomethacrylate (from Polysciences), and 8.3 mg

(0.14 mmol) TMRAHMAm (a red-fluorescent dye tethered to a polymerizable methacrylamide). The flask was sparged with nitrogen for 30 min in a sonic bath. Afterwards, 15.0 mg (65.7 mmol) APS (oxidant) was added to the flask which was subsequently immersed in an oil bath and gently warmed to 60 °C for 5 h under a nitrogen atmosphere. Excepients were removed by dialysis against water using 14-kDa MWCO dialysis tubing. The red solution of the nano-partcles failed to permeate the dialysis membrane (i.e. > ca. 10 nm). Individual particles could not be seen by light microscopy, did not settle out, or be pelletized by centrifugation at 4000 RCF. The nanoparticles were isolated by lyophilization to yield 3.393 g of red-fluorescent nanoparticles (>100 % yield, high water content). Alternatively, the nano-particles can be isolated by ultra-centrifugation. These nano-particles while invisible with conventional light microscopy were identified as distinct red-fluorescing nano-spheres using fluorescent microscopy (diameters < 1 μm).

Example 17. Synthesis of micro-beads via precipitation polymerization.

Preparation of large-porous microspheres based on HEMASucHEMA-crosslinked poly(sodiumacrylate)-co-poly(acrylic acid) and methylcellulose. A 500 mL four neck-boiling flask was charged with a magnetic stir bar, 300 mL hexane, 3.00 g methylcellulose (Mn = 40 kDa). Later, a 100 mL aqueous solution containing 39.3 g (417 mmol) sodium acrylate, 5.4 g (75 mmol) acrylic acid was added to the 500 mL flask with vigorous stirring, followed by 2.6 g (7.5 mmol) HEMASucHEMA crosslinker. Next added was a 5.6 mL (600 mg/mL) APS (oxidant) and 6.8 mL (244 mg/mL) TMED-2HCl (catalyst). A condenser was attached atop of a Dean-Stark apparatus positioned on the 500 mL reaction vessel and the contents of the flask were warmed to 62 oC for 12 hours while stirring under an N2 atmosphere. Water was removed by azeotropic distillation of hexane/water mixture. Residual was triturated with hexane and the particulate

was isolated by centrifugation and dried in vacuo. Light-microscopy (100X) revealed microspheres with diameters of ca. 50 um.

The following citations are incorporated in pertinent part by reference herein for the reasons cited in the above text.

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WHAT IS CLAIMED IS:

1. A monomeric or oligomeric cross-linker comprising a monodispersed polyacid core with at least two acidic groups being connected to reactive groups usable to cross-link polymer filaments wherein between at least one reactive group and the polyacid core is a monodispersed region degradable under aqueous conditions; the cross-linker being usable to form a network of cross-linked polymer filaments.

2. A monomeric or oligomeric cross-linker comprising a monodispersed polyacid core with at least two acidic groups connected to a monodispersed region degradable under aqueous conditions and having a covalently attached reactive group usable to form a network of cross-linked polymer filaments.

3. The cross-linker of claim 1 or 2 where the polyacid is a polycarboxylic acid.

4. The cross-linker of claim 3 described further as containing a water soluble region between at least one carboxyl group and its associated reactive group.

5. The cross-linker of claim 1 or 2 wherein the network of cross-linked polymer filaments is a hydrogel.

6. The cross-linker of claim 1 or 2 wherein the network of cross-linked polymer filaments is hydrophobic.

7. The cross-linker of claims 1 or 2 wherein the polymer filaments are water soluble.

8. The cross-linker of claims 1 or 2 wherein the polymer filaments are water insoluble.

9. The cross-linker of claim 1 or 2 wherein the polyacid core comprises at least one acidic group attached to a water soluble region that is linked to a biodegradable region having a reactive group attached thereto.

10. The cross-linker of claims 1 or 2 wherein the polyacid core comprises at least one acidic group attached to a "water insoluble" region that is linked to a biodegradable region having a reactive group attached thereto.

11. The cross-linker of claims 1 or 2 wherein the polyacid core is biocompatible.

12. The cross-linker of claim 1 or 2 wherein degradation products of the polyacid core are biocompatible .

13. The cross-linker of claim 1 or 2 where the polyacid core is a diacid.

14. The cross-linker of claim 1 or 2 where the polyacid core is a triacid.

15. The cross-linker of claim 1 or 2 where the polyacid is a pentaacid or tetraacid.

16. The cross-linker of claim 1 or 2 where the polyacid is oxalic acid, succinic acid, glutaric acid, adipic acid, fumaric acid, maleic acid, sebacic acid, malonic acid, tartaric acid, or citric acid.

17. The cross-linker of claim 1 or 2 where the polyacid is citric acid, ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA).

18. The cross-linker of claim 1 or 2 wherein cross-linked polymer filaments are separated by at least two degradable regions.

19. The cross-linker of claim 1 or 2 wherein cross-linked polymer filaments are separated by one degradable region.

20. The cross-linker of claim 1 or 2 where the degradable region comprises a hydroxyalkyl acid ester.

21. The cross-linker of claim 1 or 2 where the degradable region comprises an alpha hydroxy acid ester.

22. The cross-linker of claim 1 or 2 wherein the degradable region comprises a peptide.

23. The cross-linker of claim 1 or 2 wherein the degradable region comprises a glycolic acid ester or polyester, a DL-lactic acid ester or polyester, an L-lactic acid ester or polyester, or combinations thereof.

24. The cross-linker of claim 1 or 2 where the degradable region comprises at least one anhydride, orthoester, sulfonic acid ester, or phosphoester or combinations thereof, wherein there may be attached to one or more hydroxyalkyl acid esters.

25. The cross-linker of claim 1 or 2 wherein the degradable region comprises at least one amide functionality.

26. The cross-linker of claim 1 or 2 defined further as comprising at least one of tetraethylene glycol, diethylene glycol, triethylenetetramine, or alditol monodispersed species.

27. The cross-linker of claim 1 or 2 defined further as comprising at least one of an ethylene glycol oligomer, a poly(ethylene) glycol, a poly(ethylene) oxide, a poly(vinylpyrolidone), a poly[(ethylene oxide)-co-poly(propylene oxide)], and a poly(ethyloxazoline).

28. The cross-linker of claim 1 or 2 where the reactive group contains a carbon-carbon double bond.

29. The cross-linker of claim 1 or 2 where the reactive group is an end group.

30. The cross-linker of claim 1 or 2 wherein the reactive group is a carbonate, carbamate, hydrazone, hydrazino, cyclic ether, acid halide, acyl azide, alkylazide, succinimidyl ester, maleimide, imidazolide, amino groups, alcohol, thiol, disulfide, carbonyl, carboxylic acid, carboxylic ester, alkyl halide, aziridino, nitrile, isocyanate, isothiocyanate, phosphine, phosphonodihalide, sulfide, sulfonate, sulfonamide, sulfate, silane, or silyloxy.

31. The cross-linker of claim 1 or 2 wherein of polymer filaments are formed into cross-linked networks by thermal, catalytic, oxidation/reduction, ionizing radiation, or photochemical initiation.

32. The cross-linker of claim 1 or 2 wherein the polymer filaments are formed into cross-linked networks by pH change.

33. The cross-linker of claim 1 or 2 wherein the polymer filaments are formed into cross-linked networks by free radical addition or Michael addition.

34. The cross-linker of claim 1 or 2 where the aqueous conditions are physiological conditions.

35. The cross-linker of claims 1 or 2 where the aqueous conditions are environmental conditions.

36. The cross-linked polymer filaments of claims 1 or 2 wherein the crosslinker is biodegradable.

37. The cross-linked polymer filaments of claims 1 or 2 wherein the cross-linker is biocompatible.

38. The cross-linker of claims 1 or 2 wherein degradation products of the cross-linker are biocompatible.

39. A network of cross-linked polymer filaments formed using a monomeric or oligomeric cross-linker having a momodispersed polyacid core with at least two acidic groups connected to a covalently attached reactive group used to cross-link polymer filaments and at least one acidic group having a monodispersed region degradable under aqueous conditions between the acidic group and the reactive group.

40. The network of cross-linked polymer filaments of claim 39 formed by precipitation polymerization, dispersion polymerization or emulsion polymerization in the presence or absence of other polymerizable monomers.

41. The network of cross-linked polymer filaments of claim 39 formed by ring opening polymerization in the presence or absence of other polymerizable monomers.

42. The network of cross-linked polymer filaments of claim 39 formed by oxidation/reduction or addition/elimination polymerization in the presence or absence of other polymerizable monomers.

43. The network of cross-linked polymer filaments of claim 39 formed via condensation reaction of preformed polymer filaments and a monomeric or oligomeric cross-linker having a monodispersed polyacid core with at least two acidic groups connected to a covalently attached reactive group used to cross-link polymer filaments and at least one acidic group having a monodispersed region degradable under aqueous conditions between the acidic group and the reactive group.

44. The network of cross-linked polymer filaments of claim 39 formed via condensation reaction of preformed polymer filaments of polynucleic acids, polypeptides, proteins, and a monomeric or oligomeric cross-linker having a polyacid core with at least two acidic groups connected to a covalently attached reactive group used to cross-link polymer filaments and at least one acidic group having a region degradable under aqueous conditions between the acidic group and the reactive group.

45. The network of polymer filaments of claims 39, 40 41, or 42 further comprising biologically active molecules.

46. The network of polymer filaments of claims 39, 40, 41 or 42 comprising an organic molecule, inorganic molecule, protein, carbohydrate, poly(nucleic acid), cell, tissue or tissue aggregate.

47. A network of cross-linked polymer filaments cross-linked by a monomeric or oligomeric cross-linker comprising a central polyacid core with at least two acidic groups connected to a least one region degradable under physiological conditions, and with a covalently attached reactive end group usable to cross-link polymer filaments, the network comprising an organic radioisotope, inorganic radioisotope or nuclear magnetic resonance relaxation reagent.

48. A network of cross-linked polymer filaments cross-linked by a monomeric or oligomeric cross-linker comprising a central polyacid core with at least two acidic groups connected to at least one region degradable under environmental conditions, and with a covalently attached reactive end group usable to cross-link polymer filaments, the network

comprising an organic radioisotope, inorganic radioisotope or nuclear magnetic resonance relaxation reagent.

49. The cross-linker of claims 1 or 2 wherein the polymer filaments are biodegradable.

50. The cross-linker of claims 1 or 2 wherein the polymer filaments are biocompatible.

51. The cross-linker of claims 1 or 2 wherein degradation products of the polymer filaments are biocompatible.

52. The cross-linker of claim 1 or 2 wherein the polyacid core has a molecular weight between 30 and 2000; the degradable region has a molecular weight between 30 and 1800 and the reactive group has a molecular weight between 10 and 300.

53. A cross-linked polymeric composition containing a monomeric or oligomeric cross-linker, comprising a monodispersed polyacid core with at least two acidic groups connected to at least one monodispersed region degradable under physiological conditions, and having a covalently attached reactive group usable to cross-link polymer filaments.

54. A cross-linked polymeric composition containing a monomeric or oligomeric cross-linker, comprising a monodispersed polyacid core with at least two acidic groups connected to at least one monodispersed region degradable under environmental conditions, and having a covalently attached reactive group usable to cross-link polymer filaments.

55. A microparticle or nanoparticle polymer composition containing a monomeric or oligomeric cross-linker, comprising a monodispersed polyacid core with at least two acidic groups connected to at least one region degradable under physiological conditions, and having a covalently attached reactive group usable to cross-link polymer filaments.

56. A microparticle or nanoparticle polymer composition containing a monomeric or oligomeric cross-linker comprising a monodispersed polyacid core with at least two acidic groups connected to at least one region degradable under physiological conditions, and having a covalently attached reactive group usable to cross-link polymer filaments.

57. An article shaped as a rod, film, fiber, or other geometric shape formed from a polymer composition containing a monomeric or oligomeric cross-linker comprising a monodispersed polyacid core with at least two acidic groups connected to at least one mondispersed region degradable under physiological conditions, and having a covalently attached reactive group usable to cross-link polymer filaments.

58. An articles shaped as a rod, film, fiber, or other geometric shape formed from a polymer composition containing a monomeric or oligomeric cross-linker comprising a monodispersed polyacid core with at least two acidic groups connected to at least one mondispersed region degradable under environmental conditions, and having a covalently attached reactive group usable to cross-link polymer filaments.

59. A drug delivery system comprising a network of cross-linked polymer filaments formed using a monomeric or oligomeric cross-linker having a monodispersed polyacid core with at least two acidic groups connected to a covalently attached reactive group used to

cross-link polymer filaments and at least one acidic group having a monodispersed region degradable under aqueous conditions between the acidic group and the reactive group within which a pharmaceutically-active compound, such as an organic molecule, protein, saccaride, polysaccaride, vaccine, or polynucleic acid, is retained within the network by entrapment and/or is bound to the network by a combination of ionic, hydrophobic and hydrogen bonding interactions and/or is covalently bound to the network through a biodegradable linker, and said pharmaceutically-active compound is released from the network following in-vivo administration.

60. A medical implant or device comprising a network of cross-linked polymer filaments formed using a monomeric or oligomeric cross-linker having a monodispersed polyacid core with at least two acidic groups connected to a covalently attached reactive group used to cross-link polymer filaments and at least one acidic group having a monodispersed region degradable under aqueous conditions between the acidic group and the reactive group.

61. A superabsorbent material, such as that found in diapers, sanitary products, etc., comprising a network of cross-linked polymer filaments formed using a monomeric or oligomeric cross-linker having a monodispersed polyacid core with at least two acidic groups connected to a covalently attached reactive group used to cross-link polymer filaments and at least one acidic group having a monodispersed region degradable under aqueous conditions between the acidic group and the reactive group.

62. A tissue culture support, scaffold, or medium comprising a network of cross-linked polymer filaments formed using a monomeric or oligomeric cross-linker having a monodispersed polyacid core with at least two acidic groups connected to a covalently

attached reactive group used to cross-link polymer filaments and at least one acidic group having a monodispersed region degradable under aqueous conditions between the acidic group and the reactive group.

63. A degradable adhesive comprising a network of cross-linked polymer filaments formed using a monomeric or oligomeric cross-linker having a monodispersed polyacid core with at least two acidic groups connected to a covalently attached reactive group used to cross-link polymer filaments and at least one acidic group having a monodispersed region degradable under aqueous conditions between the acidic group and the reactive group.

64. A network of polymer filaments cross-linked by a divinylbenzene, methylenebisacrylamide or other nondegradable crosslinker monomer and one or more monomeric or oligomeric cross-linkers having a monodispersed polyacid core with at least two acidic groups connected to a covalently attached reactive group used to cross-link polymer filaments and at least one acidic group having a monodispersed region degradable under aqueous conditions between the acidic group and the reactive group.

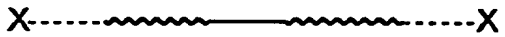
65. A network of polymer filaments cross-linked by at least two different monomeric or oligomeric cross-linkers having a monodispersed polyacid core with at least two acidic groups connected to a covalently attached reactive group used to cross-link polymer filaments and at least one acidic group having a monodispersed region degradable under aqueous conditions between the acidic group and the reactive group.

66. A network of polymer filaments cross-linked by acrylate terminated poly-alphahydroxy acids, dimethacryloyl hydroxylamine or other known degradable crosslinker

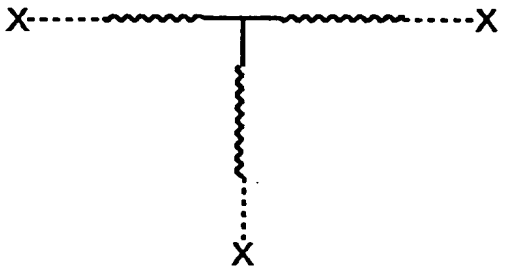
monomers and one or more monomeric or oligomeric cross-linkers having a monodispersed polyacid core with at least two acidic groups connected to a covalently attached reactive group used to cross-link polymer filaments and at least one acidic group having a monodispersed region degradable under aqueous conditions between the acidic group and the reactive group.

FIG. 1

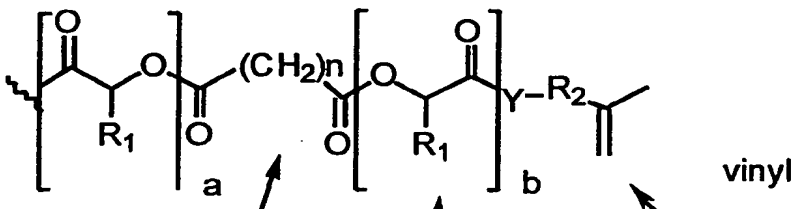
STRUCTURE A



STRUCTURE B



STRUCTURE C



Symmetrical center piece

Degradable Alpha-hydroxy ester(s)

Reactive terminal functionality

STRUCTURE D

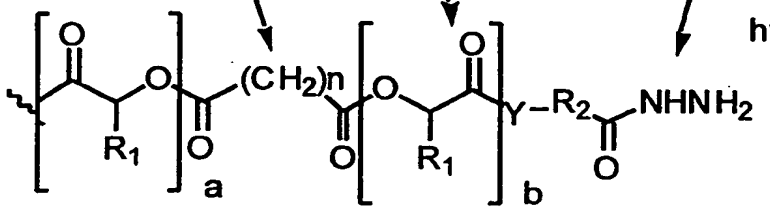


FIG. 2

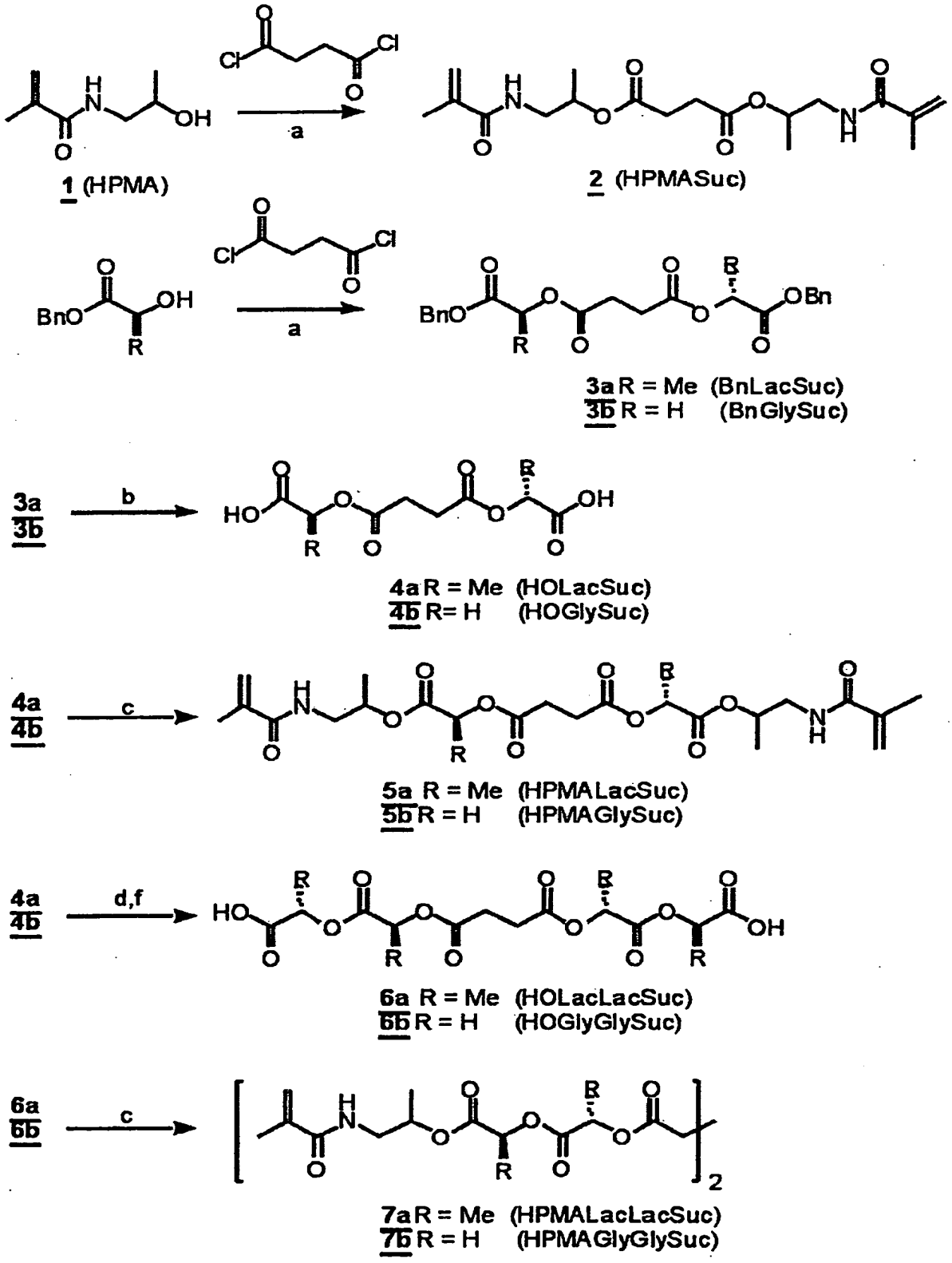

1 (HPMA)
2 (HPMASuc)
3a R = Me (BnLacSuc)
3b R = H (BnGlySuc)
4a R = Me (HOLacSuc)
4b R= H (HOGlySuc)
5a R = Me (HPMALacSuc)
5b R = H (HPMAGlySuc)
6a R = Me (HOLacLacSuc)
6b R = H (HOGlyGlySuc)
7a R = Me (HPMALacLacSuc)
7b R = H (HPMAGlyGlySuc)

FIG. 3

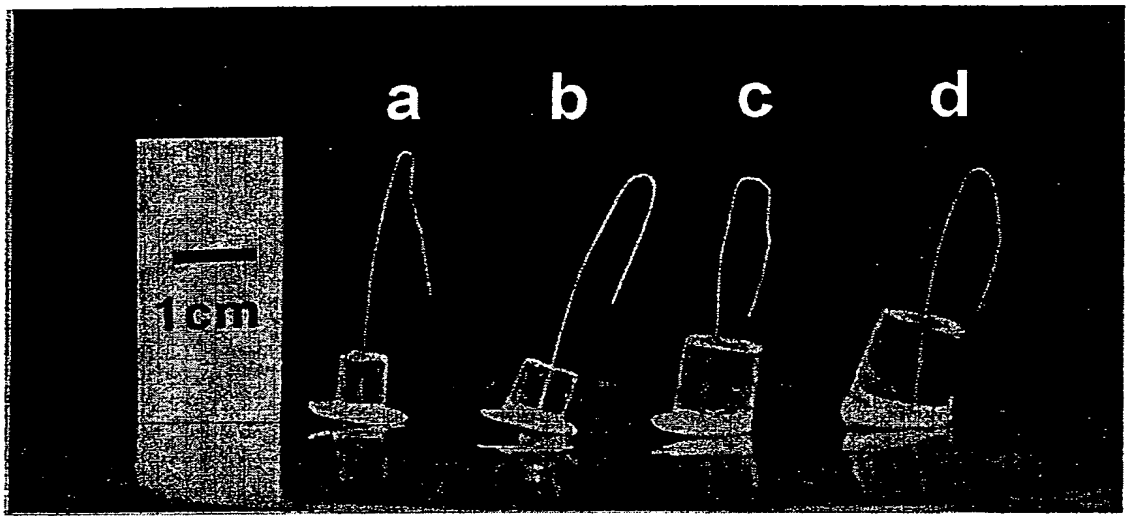


FIG. 4A

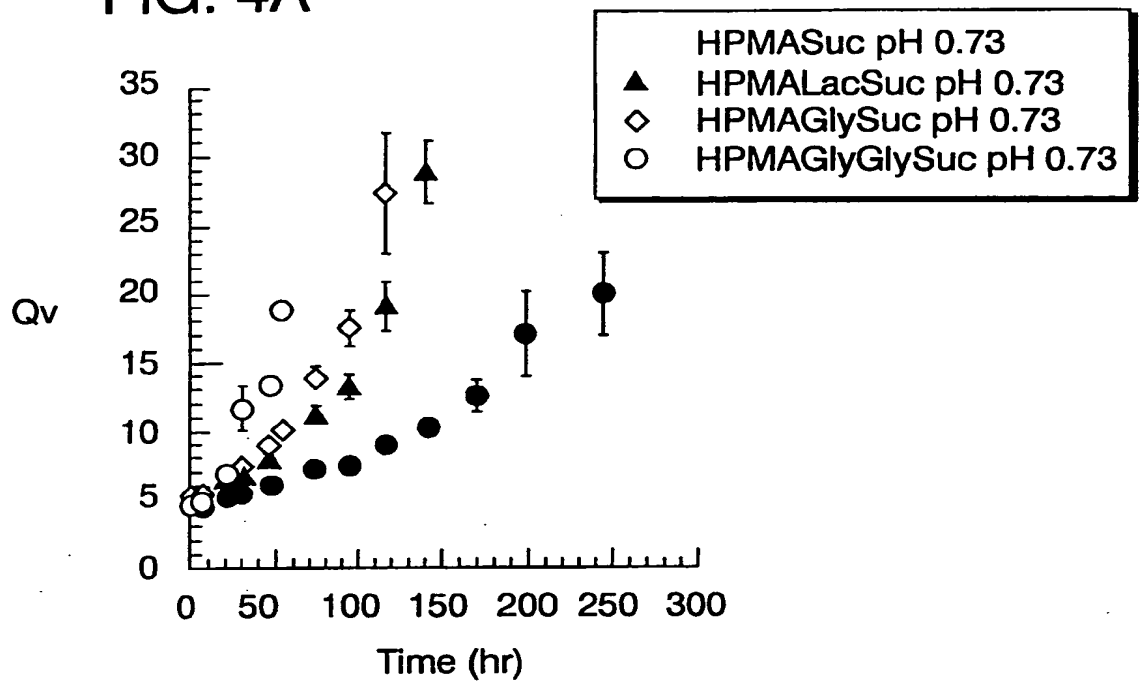

HPMASuc pH 0.73
HPMALacSuc pH 0.73
HPMAGlySuc pH 0.73
HPMAGlyGlySuc pH 0.73
Qv
35
30
25
20
15
10
5
0
0
50
100
150
200
250
300
Time (hr)

FIG. 4B

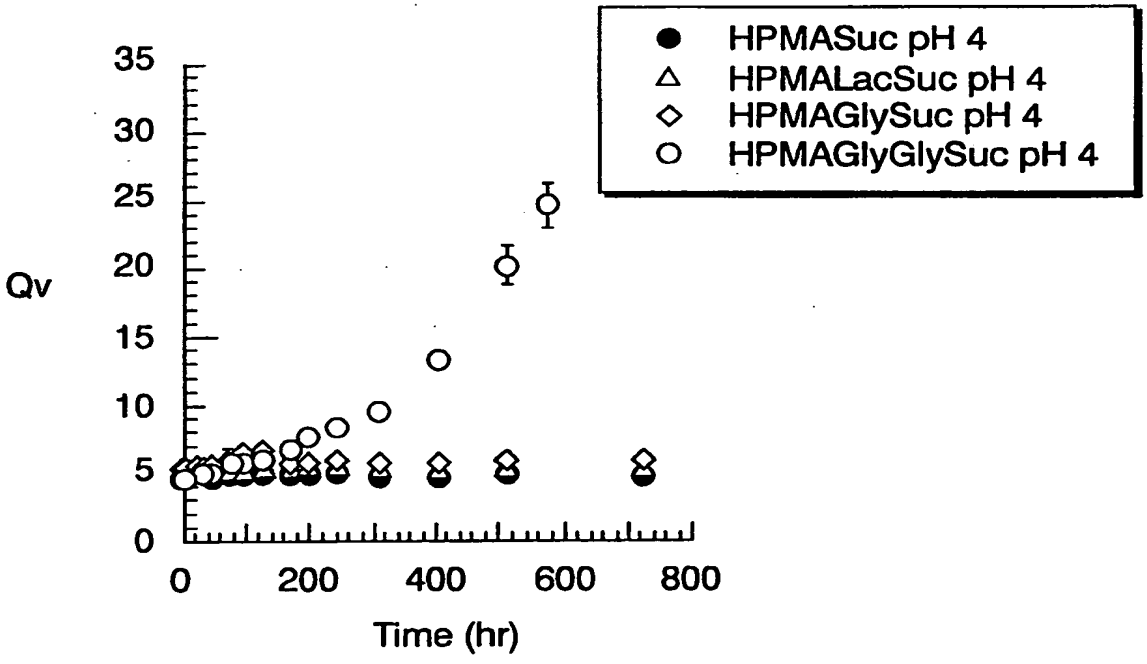

HPMASuc pH 4
HPMALacSuc pH 4
HPMAGlySuc pH 4
HPMAGlyGlySuc pH 4
Qv
35
30
25
20
15
10
5
0
0
200
400
600
800
Time (hr)

FIG. 4C

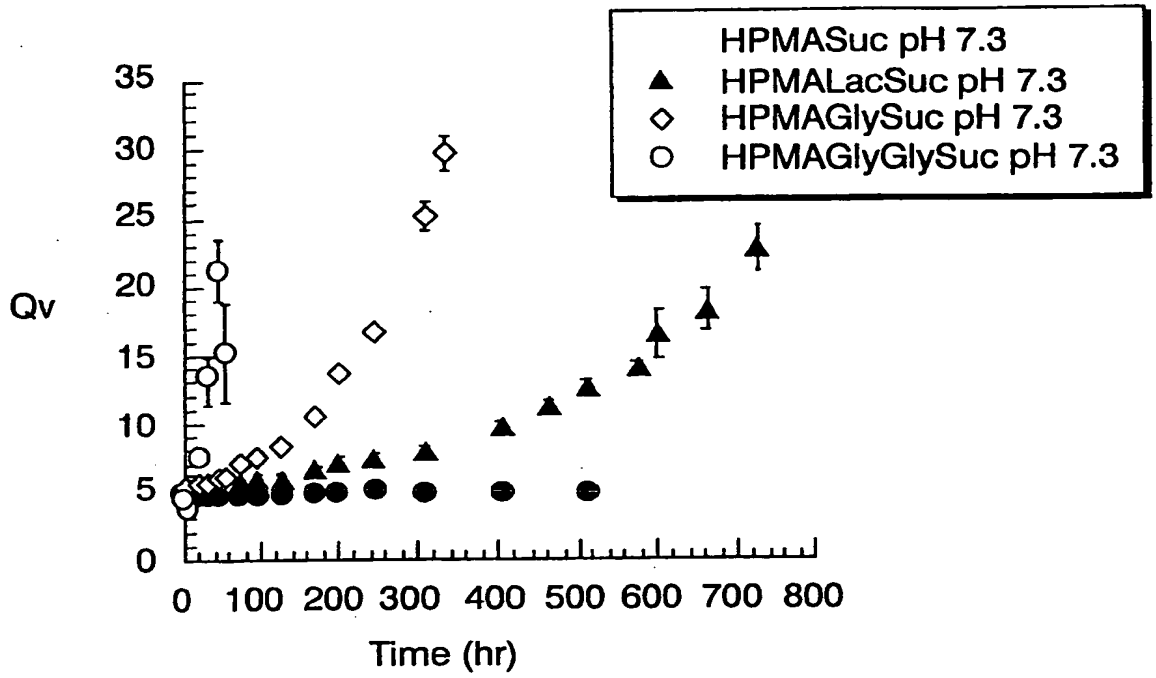

HPMASuc pH 7.3
HPMALacSuc pH 7.3
HPMAGlySuc pH 7.3
HPMAGlyGlySuc pH 7.3
Qv
35
30
25
20
15
10
5
0
0 100 200 300 400 500 600 700 800
Time (hr)

FIG. 4D

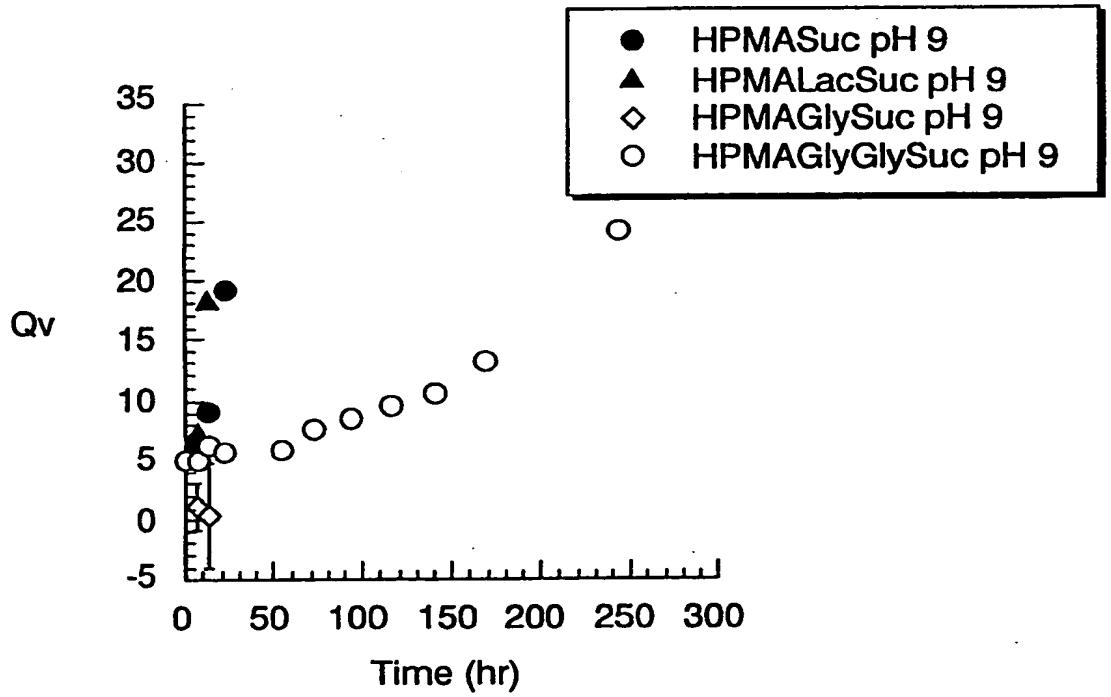

HPMASuc pH 9
HPMALacSuc pH 9
HPMAGlySuc pH 9
HPMAGlyGlySuc pH 9
Qv
35
30
25
20
15
10
5
0
-5
0 50 100 150 200 250 300
Time (hr)

FIG. 5

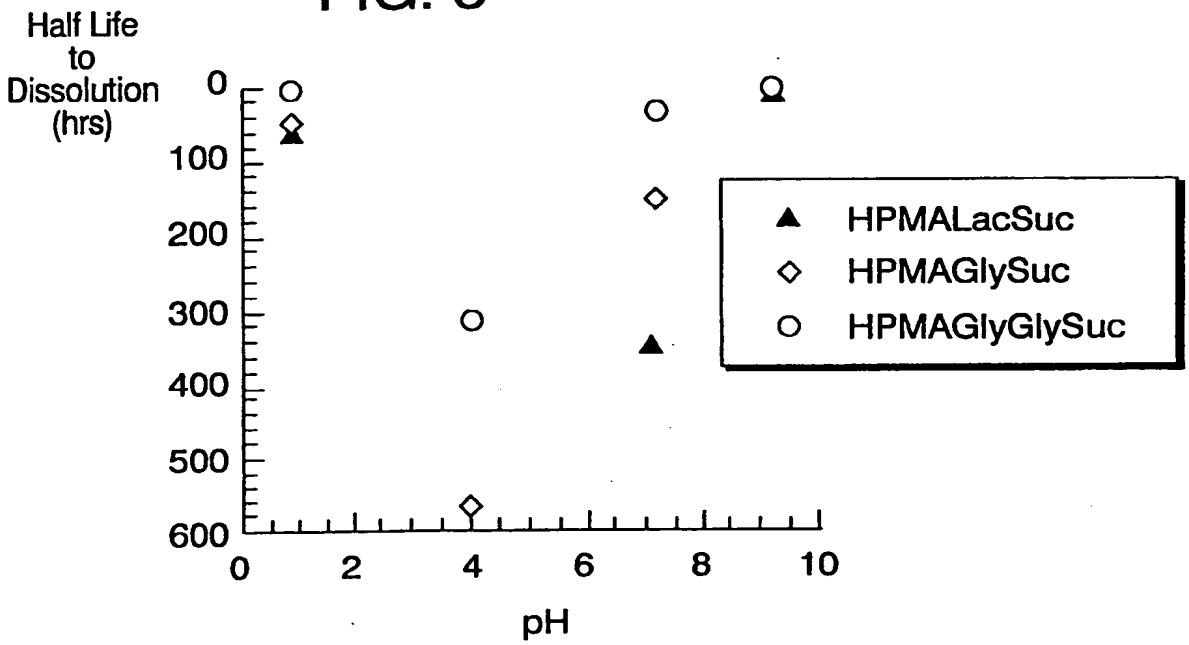

Half Life
to
Dissolution
(hrs)
0
100
200
300
400
500
600
0
2
4
6
8
10
pH
HPMALacSuc
HPMAGlySuc
HPMAGlyGlySuc

FIG. 6

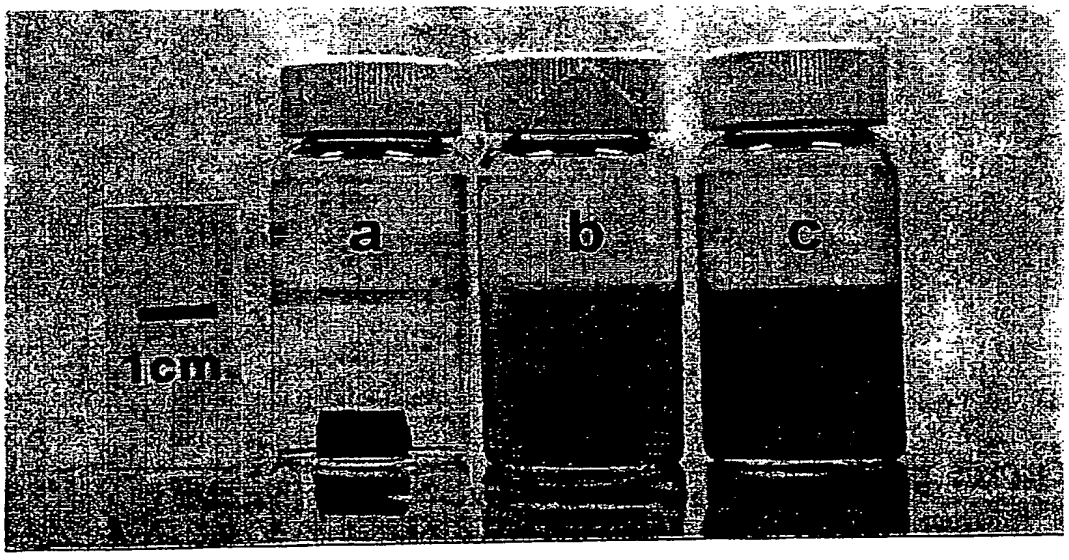

a
b
c
1cm

FIG. 7

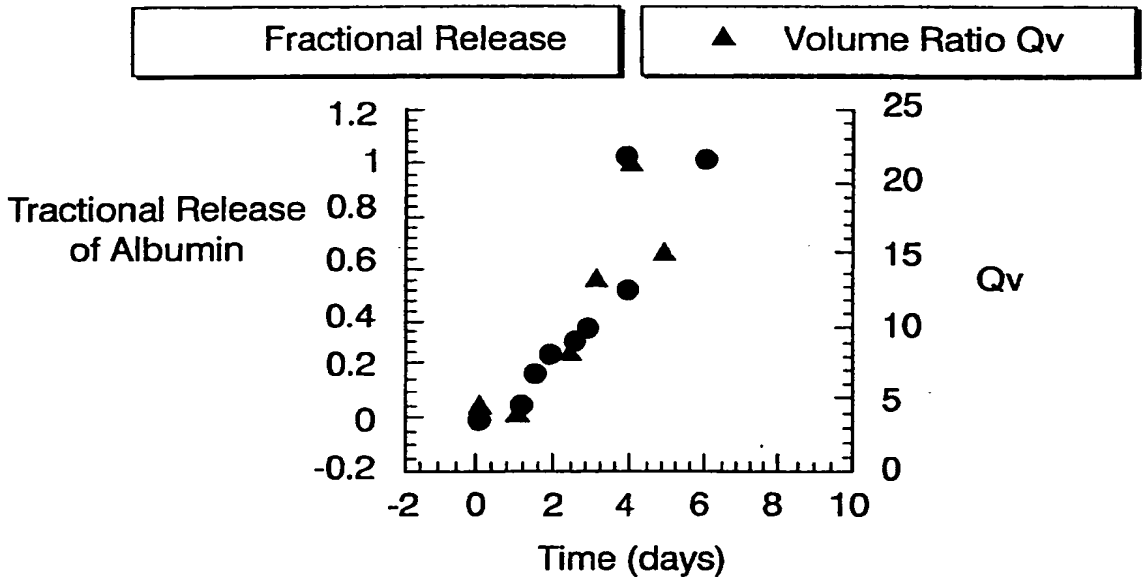
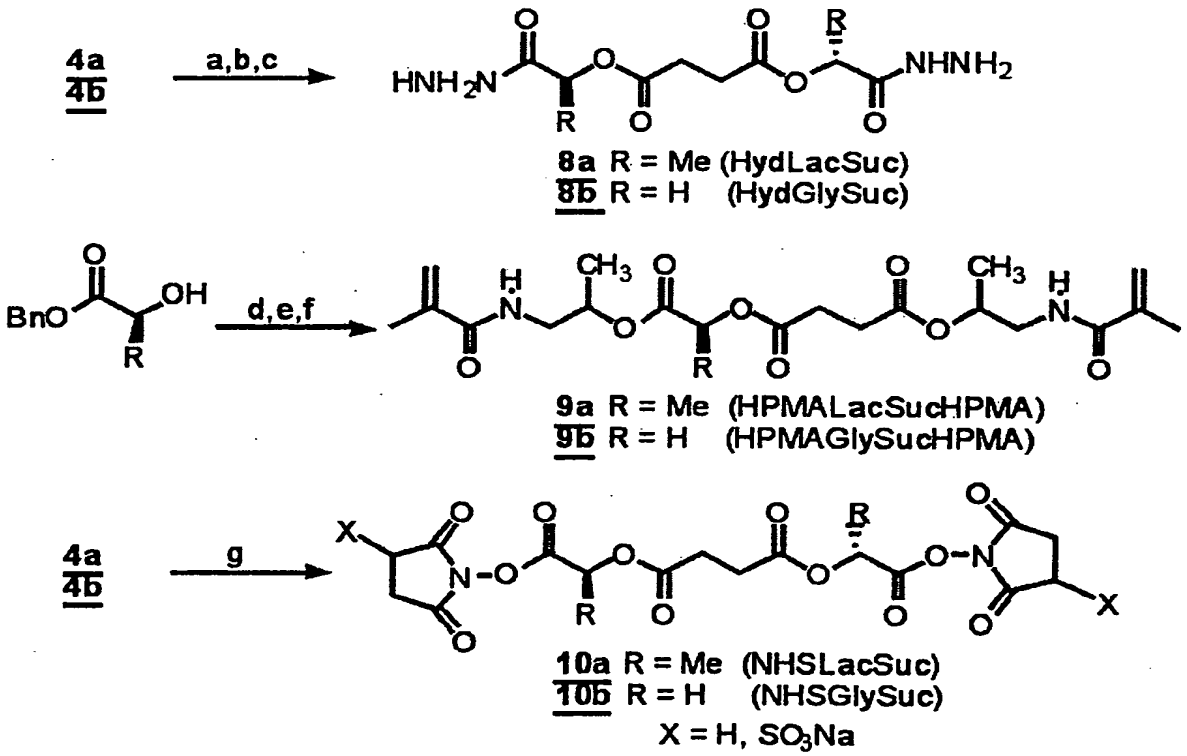

Fractional Release
Volume Ratio Qv
Tractional Release of Albumin
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
25
20
15
10
5
0
Qv
-2
0
2
4
6
8
10
Time (days)

FIG. 8


4a
4b
a,b,c
HNH2N
NHNH2
8a R = Me (HydLacSuc)
8b R = H (HydGlySuc)
BnO
OH
d,e,f
9a R = Me (HPMALacSucHPMA)
9b R = H (HPMAGlySucHPMA)
g
10a R = Me (NHSLacSuc)
10b R = H (NHSGlySuc)
X = H, SO3Na

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/US 00/17162

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C08J3/24 C08G63/00 C07C233/20 C07C243/28 C07D207/40
A61K47/00 A61K9/00 A61K49/00 A61K51/06 A61L31/00
A61F13/15

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C08K C08J C08G C07C C07D A61F A61K A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KISER, P.F. ET AL.: POLYMER PREPRINTS, vol. 41, no. 1, 22 February 2000 (2000-02-22), pages 712-713, XP000925966	1-3,5,7, 10-13, 16,18, 20,21, 23,28, 29,31, 33-40, 49-54, 57,58
P,Y	the whole document	4,9,27, 45,46, 55,59, 60,63

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2000	06/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Krische, D

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Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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